SPECIFICATIONS AND CRITERIA

for

BIOCHEMICAL COMPOUNDS

Publication No. 719.

National Academy of Sciences
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Washington D.C.
1960

~~59~~ 20 amino acids
~~carbohydrates~~
~~coenzymes~~
~~lipids~~
~~purines~~
~~pyrimidines~~
~~nucleotides~~
~~macromolecules~~

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Specifications and Criteria
for
Biochemical Compounds

Specifications and Criteria *for* Biochemical Compounds

Prepared by the

Committee on Biological Chemistry
Division of Chemistry and Chemical Technology
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SPECIFICATIONS AND CRITERIA FOR BIOCHEMICAL COMPOUNDS

INTRODUCTION

This publication is the result of a program under way for over five years to improve the quality of chemicals available for biochemical research by establishing criteria, standards, or specifications which can be used for describing such chemicals, particularly with regard to the aspect of purity. These pages represent an initial effort to satisfy a long-felt need of biochemists for more knowledge of the chemicals used in their investigations.

The program was initiated by Dr. Sam R. Hall[1] who, as a result of discussion with prominent biochemists, brought this matter to the attention of the Biochemistry Study Section of the National Institutes of Health at its meeting of January 1955. The Study Section recommended that a joint committee to study the problem be established by the two largest organized groups of biochemists, the American Society of Biological Chemistry and the Division of Biological Chemistry of the American Chemical Society. The governing bodies of these societies referred the matter to the then newly-formed Committee on Biological Chemistry of the Division of Chemistry and Chemical Technology, National Academy of Sciences-National Research Council. Under the chairmanship of Prof. Herbert E. Carter, this Committee surveyed the need by submitting a questionnaire to the membership of both societies. The response was 42% (considerably beyond normal expectations) and about 90% of the replies indicated a serious need for improvement in the quality and the standards of biochemicals.[2] This response, together with many additional comments and communications, was regarded by the Committee on Biological Chemistry as a "mandate for action."

It was recognized that rigid standard specifications, supplemented with preparation of standard reference substances, would be desirable. However, a review of the experience of the American Chemical Society and the United States Pharmacopeia in similar undertakings convinced the Committee that such rigidity would result in a task of such magnitude as not to be immediately feasible. The Committee felt that a more modest, but immediate, first step that would go far toward meeting the needs of users of biochemicals would be issuance of description sheets outlining the characteristics of the highest grades of the more common biochemicals commercially available, such sheets to provide information on the sources, methods of preparation, common physical properties, likely impurities and

[1] Dr. Hall was at that time Executive Secretary of the Endocrinology Study Section of the National Institutes of Health. He played a leading role in the early development of this program and remained active in it until he left NIH in 1956 to join the staff of the American Cancer Society.

[2] *Science*, **123**, 54 (1956).

methods for their determination, methods of assay, stability, etc. The selection of criteria for such descriptions was delegated to subcommittees of experts in universities, research institutes and industry. The membership of these subcommittees is listed after this Introduction and identifies the workers who have done the sometimes wearisome spadework in compiling these initial criteria sheets.

The principle guiding the Committee and its subcommittees has been selection of specifications or criteria which will permit an adequate characterization of each subject biochemical. These criteria are not idealized[3], but are known to be met by the best commercial preparations. The cooperation of commercial suppliers in formally characterizing their products in relation to the values presented is on a voluntary basis.

The subcommittees are aware that fine biochemicals—unlike inorganic chemicals—are supplied by a comparatively small number of suppliers who are, however, widely dispersed on this continent, as well as in Europe, Asia and South America. Their preparations may involve not only a variety of procedures, but also starting materials of varying degrees of purity. Some classes of compounds, such as the sugars and some of the amino acids, were already well characterized before this program was initiated. For those compounds, the sheets represent accurate descriptions of existing high-grade commercial preparations. Many other compounds, however, such as the enzymes, coenzymes, and certain purine and pyrimidine derivatives, cannot be described so rigorously. The subcommittees felt that it would be more appropriate to describe these substances in terms of criteria by which the user could form his own judgment as to their purity.

The Committee believes that the major burden of evaluating the acceptability of biochemicals for any specific use or purpose belongs, in the long run, on the users themselves. Although the representations of suppliers may generally be relied upon, users should be acquainted with the best available and practicable guide for recognizing the degree of purity of biochemicals which they require, and it should be the user's responsibility to determine what, if any, further purification may be needed.

This publication represents only a beginning. Each of the subcommittees has under consideration further lists of compounds and, as specifications and criteria are compiled, Supplements will be issued from time to time. Other subcommittees may be appointed to study additional categories of biochemicals.

All of the specifications and criteria in this publication were formulated with the aid of suppliers' representatives and were submitted for review

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[3] Some members favor supplementary specifications and criteria representing the highest state of purity theoretically attainable, established scientifically and without regard to demand, cost of production, or other economic factors. This aspect was deferred for consideration at some future time.

to known suppliers. These specifications and criteria represent the best manufacturing practice to date. In the future—in some cases, in the very near future—improved techniques and increased knowledge will make possible production of some biochemicals with a degree of purity not now attainable. The sheets for those biochemicals will nevertheless remain useful as a basis for characterization but will, of course, no longer represent the best of such substances that are obtainable commercially. The Committee intends to issue amended sheets that will reflect such advances in "the state of the art."

The Committee on Biological Chemistry would greatly appreciate having any errors or omissions found in this publication brought to its attention. This request is earnestly directed to all subscribers and any other interested persons.

The Committee is also eager to receive any helpful criticisms and suggestions regarding either substantive text or publication format. As with many new ventures with multiple authorship, there have been honest differences of opinion on selection of information and on the best manner to assemble and present the material. It is hoped that suggestions from the scientific public will result in improvement of future editions.

Another important aspect is evaluation by users of the adequacy of the data in actual situations. If some users find, for example, that the criteria sheets are of limited value to them because of the manner of presentation or because certain additional information is not supplied, these facts will be of interest to the Committee and communications will be welcome. In this connection, perhaps it should be pointed out that the biochemical characterizations were developed for general use. Those users with special requirements (such as the absence of specific trace elements, growth factors, and other components) should discuss such needs directly with the manufacturers.

Reports of errors, suggestions for revisions, and other comment on either substance or format may be addressed to:

Committee on Biological Chemistry
Division of Chemistry and Chemical Technology
National Academy of Sciences-National Research Council
Washington 25, D. C.

In conclusion, the Committee wishes to express appreciation for encouragement and support by the National Institutes of Health whose PHS Grant No. RG-5709-DGMS has, in large part, made possible the compilation and publication of *Specifications and Criteria for Biochemical Compounds.*

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AMINO ACIDS

General Remarks and Analytical Procedures

The Subcommittee on Amino Acids has selected for consideration those amino acids which for the most part are readily available on the commercial market. Of these compounds, some are racemic and some are the natural, optically-active L-forms. The latter compounds have been isolated from the hydrolysates of various proteins or by resolution of the synthetic, racemic amino acids.

The manufacturers of amino acids have performed an outstanding service in furnishing materials to the biochemical field in volume and at relatively low cost. Each year sees a lowering of costs with consequent gain to the consumer. In general, the quality of the amino acids sold has been high, and for this the manufacturers deserve added credit. What constitutes an adequate standard of purity, however, is in the long run the responsibility of the investigator.

The Subcommittee has attempted to describe methods which will enable the investigator, with the usual equipment available in the modern laboratory, to identify, to establish the degree of purity, and to employ some routine methods of purifying the amino acids listed. The Subcommittee has selected only those amino acids which are generally recognized to be present in proteins for consideration at this time. Although some of these amino acids are available as the D-forms, they have not been considered in this initial publication.

Analytical and Identification Procedures[1, 2]

A. *Melting Point:*

The melting points of the amino acids and their salts are generally not sharp and therefore of little value as a means either of identification or as a criterion of purity.

B. *Loss of Weight on Drying:*

Unless otherwise specified, the general procedure for determining volatile contaminants (normally water) is the following, described in section 29.6 ("Vacuum Drying—Official") of the *Official Methods of Analysis of the Association of Official Agricultural Chemists*, AOAC, 8th ed., Washington, D. C., 1955, p. 532. Dry 2–5 g. of a representative sample (powdered, if necessary) in a flat dish (Ni, Pt, or Al) with tight-fitting cover, 2 hrs. at not over 70°C. (preferably 60°C.), under reduced pressure (not exceeding 50 mm. of Hg). Remove the dish from the oven, cover, cool in a desiccator, and weigh. Redry 1 hour and repeat process until change in weight between successive dryings at 1 hour intervals is not more than 2 mg.

Unless it is otherwise indicated, the pure amino acids should not lose more than 0.5% of their weight.

Note: Bleed oven with current of dry air during drying to insure remov of H_2O vapors.

C. *Ultimate Analyses:*

These are performed by standard procedures. The values found shou agree with the calculated values within the following limits:

C 1% relative, N 3% relative and sulfated ash 0.1%.

D. *Specific Rotation:*

The specific optical rotations are determined on samples previously drie to constant weight by the procedure given in section B and made up to 1–2 per cent solution. The numerical value obtained will depend upon th solvent system employed according to the rule of Clough, Lutz, an Jirgenson. This rule applies only to those α-amino acids which contain single center of asymmetry and may be given as follows: The addition o acid to an aqueous solution of an L-amino acid will cause the specific rota tion to be shifted toward a more positive direction; aqueous solutions o D-amino acids show a negative shift in direction or rotation on like treatment

The above rule is not rigidly applicable to α-amino acids with two o more centers of optical asymmetry. It does become applicable, however when based upon shifts in the partial molar rotation of the α-asymmetri center as determined by calculations developed by Winitz, Birnbaum, and Greenstein, *J. Am. Chem. Soc.*, **77**, 716 (1955).

$[\alpha]_D^t = \frac{100\theta}{cL}$ where $[\alpha]_D^t$ is the specific rotation at t degrees centigrade

using the D-line of sodium.

θ is the observed rotation in degrees.

c is the concentration in gm. of amino acid per 100 ml. of solution.

L is the length of the polarimeter tube in decimeters, and

t is the temperature.

The D-line of sodium is used except where indicated otherwise. Reading should be taken in a darkened room, preferably by two or more individuals The method is not of the highest accuracy, and the results are simply thos agreed upon by the majority of observers. There can well be an allowance of $\pm$ 2 per cent in such readings. A large excess of HCl is necessary i most instances, in order that the amino acid be present as a single species

E. *Paper Chromatography:*[3, 4]

Although the paper chromatographic methods described here will no give the quantities of impurities present, the latter can be approximated by noting the size and intensity of the spots in comparison with those given by known quantities of the contaminant. The sulfur-containing amino acids, and the iodotyrosines and iodothyronines may decompose during and after application to the paper. It is, therefore, advisable to avoid the

use of heat and strong light during their application to the paper and to develop the chromatograms immediately after the spot has dried. The following solvents and staining systems are used:

Solvents

1. 88% w/v Phenol:H_2O = 100:20 v/v. Add 15 mg. per cent of 8-hydroxyquinoline. Run in the presence of 100 ml. of 2 *N* NH_4OH.
2. 1-Butanol: Acetic Acid: H_2O = 450:50:125 v/v.

3a. 2-Butanol: 3.3% aq. NH_3 = 150:60 v/v. Aqueous NH_4OH is prepared by diluting 65 ml. of 14.8 *N* NH_4OH to 500 ml. with H_2O.

3b. 2-Butanol: 3% aq. NH_3 = 150:50 v/v (1:6 aq. NH_4OH atmosphere).

A. Solvent 1 followed by Solvent 2.

B. Solvent 1 followed by Solvent 3.

C. Solvent 2 followed by Solvent 3.

Paper: Whatman No. 1 and/or Whatman No. 3.

Color Reagents

Ninhydrin for all amino acids: 0.25% w/v in acetone.

Sakaguchi for arginine: α-Naphthol: hypobromite reagent. Prepare a 0.01 per cent w/v solution of α-naphthol in ethanol containing 5 per cent w/v urea. Add KOH to 5 per cent w/v just before spraying. Air dry a few minutes, and spray lightly with a solution of 0.8 ml. of Br_2 in 100 ml. of 5 per cent w/v KOH.

Nitroprusside for cystine and cysteine: Reagent 1: Sodium nitroprusside (1.5 g.) is dissolved in 5 ml. of 2 *N* H_2SO_4. Then 95 ml. of methanol and 10 ml. of 28% ammonia are added. The solution is filtered and stored in the refrigerator. Reagent 2: Two grams of NaCN are dissolved in 5 ml. of water and diluted to 100 ml. with methanol. Prepare fresh.

Pauly for histidine and tyrosine: Reagents: sulfanilamide (1% w/v) in 10% v/v HCl; 5% w/v $NaNO_2$; half-saturated Na_2CO_3. Place 5 ml. of sulfanilamide solution and 5 ml. of $NaNO_2$ in a 100 ml. separatory funnel. Shake for 1 minute. Then add 50 ml. of 1-butanol. Shake for 1 minute and let stand for 4 minutes. Decant the butanol layer, and spray or dip the chromatogram. Dry the sheet in a current of air and then dip into the Na_2CO_3 solution. Imidazoles give a deep cherry red color.

Ehrlich for tryptophan: A mixture of 1 g. of *p*-dimethylaminobenzaldehyde, 90 ml. of acetone and 10 ml. of concentrated HCl is freshly prepared.

Platinic iodide for cystine, cysteine and methionine: Add in the following order 4 ml. of 0.002 *M* $PtCl_6$, 0.25 ml. of 1 *N* KI, 0.4 ml. of 2 *N* HCl, and 76 ml. of acetone. The dried chromatograms are dipped into this reagent. Cystine, cysteine, methionine and some reducing substances also give a white spot on a red-purple background.

Ceric sulfate: sodium arsenite for iodoamino acids (see directions for iodo-compounds below).

Periodate : Nessler for serine and threonine: The paper is sprayed with Nessler's reagent almost saturated with $NaIO_4$.

Isatin for proline and hydroxyproline: Spray with 0.40% w/v isatin in acetone and, after drying in air, the chromatogram is heated for 10 minutes in an oven at 70–76°C. and 100% humidity. Proline and hydroxyproline give blue colors; cystine and tyrosine often also give blue colors. Glutamic and aspartic acids give pink spots which turn blue on standing. The other amino acids give pink spots which fade. Pipecolic acid (piperidine-2-carboxylic acid) also gives a blue color with isatin.

Qualitative Detection of Iodo-Compounds on Paper Chromatograms

Reagents: A. 10 per cent ceric sulfate ($Ce(HSO_4)_4$) w/v in 10 per cent H_2SO_4 v/v.

B. 5 per cent w/v aqueous sodium arsenite (Na_2AsO_3).

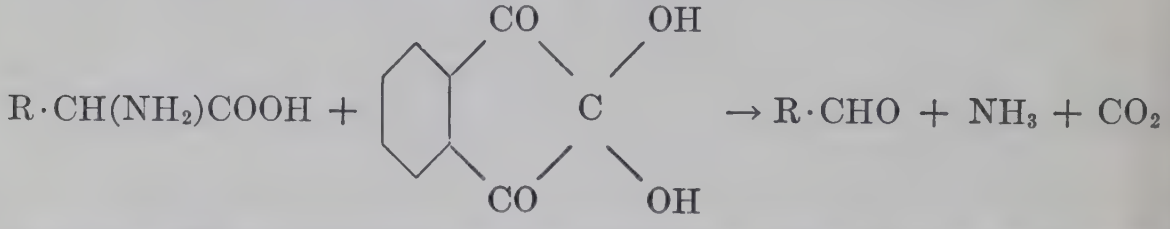
C. 0.05 per cent w/v methylene blue.

D. Ammonia (sp. gr. 0.90).

Procedure: After development of the chromatogram, the paper is dried thoroughly in an atmosphere free of phenol. The paper is then sprayed lightly on both sides with a mixture of Reagents A and B in the proportions of 2 and 3 volumes respectively (use reagent immediately after mixing). The paper is dried in an air stream (fan) for 5 minutes. It is then sprayed lightly with Reagent C, dried for 5 minutes and then subjected to NH_3 vapor. The completion of the neutralization can be detected by a change in the background color from pale purple to bright yellow. The chromatogram is again dried. The iodo-compounds appear as bright blue spots against a yellow background. The minimum amount detectable is in the order of 0.1 to 0.2 micrograms of iodine.

F. *Estimation of Carboxyl Groups* (Van Slyke):

Principle: Amino acids are oxidized at acid pH to yield CO_2 which is determined by collection in barium hydroxide.



Apparatus: Carbon dioxide can be determined either in the well-known Van Slyke-Neill manometric gas analysis apparatus or more conveniently in two 25 ml. Erlenmeyer flasks without lips, one for the reaction and one for standard $Ba(OH)_2$. The flasks are connected to each other by means of a U-tube. The U-tube has a small sidearm which permits the entire apparatus to be evacuated.

Reagents: Citrate buffers. pH 4.7: grind together 17.65 gm. of trisodium citrate dihydrate and 8.40 gm. of citric acid monohydrate to a fine powder.

pH 2.5: grind together 2.06 gm. of trisodium citrate dihydrate and 19.15 gm. of citric acid monohydrate.

Standard Ba(OH)₂. 0.25 N: saturated $Ba(OH)_2$ is adjusted to 0.3 *N* with CO_2-free water. Five volumes of this solution are mixed with 1 volume of 12% w/v $BaCl_2$ prepared in CO_2-free H_2O. Three ml. of the standard baryta should be titrated with *N*/7 HCl to pH 8 using the veronal buffer color standard with 1 drop of 1% phenolphthalein as the indicator to obtain the blank value.

Veronal buffer. pH 8: Dissolve 10.3 gm. of sodium veronal in 500 ml. of water. Mix 7 ml. of this solution with 4 ml. of *N*/14 HCl, add phenolphthalein. This is the color to which the $Ba(OH)_2$ is titrated.

Method: 1. *Hydrolysate.* Adjust to faintly acid to bromphenol blue.

2. *Removal of Preformed CO_2.* To 1 to 5 ml. of solution in the 25 ml. Erlenmeyer flask add 50 to 100 mg. of either pH 2.5 or 4.7 citrate buffer and 1 drop of caprylic alcohol. Boil off preformed CO_2. Stopper and cool to 15°. In the meantime remove CO_2 from titration flask by a stream of CO_2-free air. Add 3 ml. of 0.25 *N* $Ba(OH)_2$ to the titration flask.

3. *Oxidation.* Add 50 to 100 mg. of ninhydrin to reaction flask. Connect to $Ba(OH)_2$ flask by means of U-tube and rubber tubing. Make connections glass to glass. Evacuate at the water pump and then immerse entire apparatus as far as the top of the U-tube in boiling water for seven minutes or longer.

4. *Distillation of CO_2.* Cool the $Ba(OH)_2$ flask in water but keep the reaction vessel at 100°. Shake for three minutes to facilitate the distillation and absorption of all CO_2.

5. *Titration.* Titrate $Ba(OH)_2$ with *N*/7 HCl from a 5 ml. burette using 1 drop of 1% phenolphthalein as the indicator. Subtract reagent blank.

1 ml. *N*/7 HCl = 1 mg. of carboxyl nitrogen

G. *Estimation of Amino Group (Van Slyke Modified by Sobel):*

Principle: Ammonia resulting from the oxidative deamination of amino acids with ninhydrin is aerated into boric acid and titrated.

Reagents: 1. *Caprylic alcohol* saturated with thymol.

2. *Ninhydrin*, solid.

3. *Buffer:* pH 2.5 citrate buffer (*cf.* section F). Dissolve in water to 10% w/v.

4. *H_2O_2:* 30% w/v.

5. Saturated *KOH:* To a cylinder containing water under mineral oil 1 inch thick, add solid KOH to saturation. Preserve under oil.

6. *Indicator:* Ten parts of 0.1% w/v bromcresol green plus 1 or 2 parts of 0.1% w/v methyl red in 95% ethanol.

7. *Boric Acid:* Twenty gm. of H_2BO_3 are diluted to 1 liter with water. Add 20 ml. of indicator per liter.

8. *Hydrochloric Acid:* Standard 0.0714 *N* HCl.

Method: 1 ml. of solution containing 20 to 100 mg. of N is placed in an aeration tube, add 0.3 ml. of buffer and 50 mg. of ninhydrin. The pH should be 2.4 to 2.6. Mix and place in boiling H_2O for 10 minutes. At end of two minutes heating, shake to dissolve the ninhydrin. At the end of 10 minutes, add 3 drops of 30% w/v H_2O_2, shake and heat three minutes longer.

Set up the tubes for aeration. Add 1 ml. of saturated KOH and aerate into the boric acid solution for 40 minutes using 1.5 ml. of 2% boric acid to trap the NH_3. Titrate with HCl.

$$\text{ml. of 0.0714 } N \text{ HCl} \times 1000 = \text{mg. of amino acid N}$$

H. *Estimation of Amino Group (Titration):*[5]

Principle: The amino group is titrated with an acetic acid solution of perchloric acid in glacial acetic acid.

Reagents: 1. Perchloric acid, approximately 0.2*N*. Dissolve 17.3 ml. of 70% w/v perchloric acid in 800 ml. of glacial acetic acid, add 44 gm. of acetic anhydride and dilute to 1 liter with glacial acetic acid. Allow to stand overnight and then standardize an aliquot against 250.0 mg. of pure dry Na_2CO_3 (see below). *Do Not Heat!*

2. Bromothymol blue indicator. 0.1% w/v in glacial acetic acid.

3. Glacial acetic acid, reagent grade.

Method: Weigh 1.000 gm. of amino acid into a 150-ml. beaker and add 25 ml. of glacial acetic acid and 10 drops of indicator. Titrate with 0.2 *N* perchloric acid with constant (mechanical) stirring to a faint pink color.

Calculations:

$$\frac{\text{Na}_2\text{CO}_3 \text{ in gm.}}{0.0530 \times \text{ml. of HClO}_4} = \text{normality of HClO}_4$$

$$\frac{\text{ml. of HClO}_4 \times N \times \text{M.W. of Amino Acid} \times 100}{\text{sample weight in gm.}} = \% \text{ of amino acid}$$

Comment: As the $HClO_4$:CH_3COOH mixture has a high coefficient of expansion, it should be standardized each time that it is used.

I. *Estimation of Carboxyl Group (Titration):*[6]

Principle: The carboxyl group is titrated with NaOH in the presence of HCHO.

Reagents: Sodium hydroxide, 1 *N* free of carbonate.

Formaldehyde: 35 to 37%, reagent grade.

Method: Dissolve 2 to 3 gm. of amino acid in 100 ml. of HCHO and titrate with 1 *N* NaOH using a pH meter to follow the reaction. The end-point is that addition of 0.1 ml. of NaOH which causes a very marked increase in pH. Correct for the reagent blank by titrating 100 ml. of HCHO to the same pH as the end-point.

Calculation:

$$\frac{\text{net ml. of } N \text{ NaOH} \times \text{M.W. of Amino Acid} \times 100}{\text{sample weight in gm.}} = \% \text{ of amino acid}$$

J. *Infrared Spectroscopy:*

Infrared spectra can be used for the identification of amino acids and are also useful in specific cases for the determination of small amounts of impurities that are difficult to detect by paper chromatography. Examples (with appropriate absorption bands of the impurity) are: a) DL-leucine (11.78 μ) in the epimeric mixture of L-isoleucine with D-alloisoleucine; b) the epimeric mixture of L-isoleucine and D-alloisoleucine (12.48 μ) in DL-leucine, and c) DL-allothreonine (9.95 μ or 11.99 μ) in DL-threonine. Minimum detectable amounts are about 0.5% in the leucine-isoleucine pairs and about 2% of allothreonine in threonine.

K. *Ultraviolet Absorption:*

Tyrosine and tryptophan possess characteristic absorption in the ultraviolet region. The molecular extinction coefficients for tyrosine in acid are 1340 at 274.5 mμ and 8200 at 223.0 mμ, both of which are maxima, and 170 at 245.0 mμ which represents a minimum in the curve for this region; the corresponding values in alkaline solution are 2330 at 293.5 mμ and 11,050 at 240.0 mμ for the maxima and 1000 at 269.5 mμ for the minimum. For tryptophan in either acid or alkaline solution, the maxima are 4550 and 5550 at 287.5 mμ and 278.0 mμ, respectively, and the minimum 1930 at 242.0 mμ.

L. *Determination of the Optical Purity of Amino Acids with* L- *or* D-*Amino Acid Oxidase and Bacterial Decarboxylases:*

Most of the amino acids isolated from proteins can be obtained in the pure L-form. L-Cystine, however, is a notable exception, for it is often not only contaminated by L-tyrosine (removable by the charcoal treatment in the recrystallization procedures), but also by D- and *meso*cystine as a result of some racemization by the boiling HCl used in the hydrolysis of the original protein. Experience in several laboratories has shown commercially-available L-cystine to contain from 5 to 15% D-cystine.

Contamination by the D-isomer in samples of supposedly pure L-isomers of cystine, hydroxyproline, proline, leucine, phenylalanine, tyrosine, etc., can be determined using D-amino acid oxidase as follows: One thousand micromoles of the isomer to be tested are added to each of four Warburg vessels and to two of these flasks 1 micromole of the optical enantiomorph is also introduced. To these four flasks, as well as to two others which will serve as enzyme blanks, 1.5 to 2.5 ml. of buffer solution are added. The buffers are made up of 0.1 *M* sodium pyrophosphate at pH 8.1 for use with D-amino acid oxidase, 0.1 *M* "tris" buffer at pH 7.2 with L-amino acid oxidase, and 0.1 *M* acetate buffer at pH 4.9 with the bacterial decarboxylases.

Twenty-five units of crystalline catalase are added to the main compartment of the Warburg vessels in the determinations with L-amino acid oxidase. There is usually sufficient catalase present in the crude D-amino acid oxidase preparations, so that no further addition of this enzyme is required. Three hundred to 400 μl. of the enzyme solutions are placed in the side arms of all six vessels. The solutions of hog kidney D-amino acid oxidase, snake venom L-amino acid oxidase, and the pertinent L-specific bacterial decarboxylases are used at almost saturation levels. After a 10- to 15-minute equilibrium period the flasks are tipped and the gas evolution or consumption is noted from time to time until the reaction has ended (15 to 120 minutes). Ten micromoles of an optically pure, completely susceptible amino acid consumes 112 μl. of oxygen gas or evolves 224 μl. of carbon dioxide gas, under standard conditions. This method is obviously applicable only where 1 micromole of added susceptible isomer is readily and quantitatively oxidized or decarboxylated in the presence of the 1000-fold amount of the resistant enantiomorph.

The 1000 micromoles of the L-isomer alone should consume less than 1 microatom of O_2, while the added 1 micromole of D-isomer should be quantitatively oxidized as shown by the increment in the value over the L-isomer. All values are corrected for the enzyme blanks.

M. *Recrystallization:*

Many of the amino acids are easy to purify for they are readily and rapidly crystallizable either from water or from water-ethanol mixtures. For this reason, the subcommittee has described, where possible, the appropriate solvent required for each of the amino acids. The general, but not invariable, procedure is to bring the solvent to the boiling temperatures and to pour it over the solid amino acid in a large Erlenmeyer flask. Enough of the hot solvent is added to bring most of the amino acid into solution. The mixture is then placed on the hot plate and gently boiled. If the remainer of the solid does not go into solution, more of the hot solvent is added to the boiling mixture until practically all of the solid is dissolved. At this point, acid-washed charcoal (Norit or Darco) is carefully added (if added too quickly frothing will occur) in proportion of about 1 gram of charcoal for every 100 grams of amino acid, and the mixture is boiled carefully for 2 to 5 minutes. At the end of this period, the mixture is filtered through *two* layers of filter paper (usually Whatman No. 4) in a hot water jacketed funnel. The hot filtrate may then be chilled without further treatment (proline, tryptophan, etc.), or a second hot solvent may be added slowly and with careful stirring to some specified volume (alanine, arginine, etc.) or a reagent such as hot dilute ammonium hydroxide is introduced (cystine) prior to chilling. In all cases, the amino acid is obtained in crystalline form within a few hours, filtered off with suction, washed successively with ethanol and ether, and dried. The recoveries are generally better than

80 per cent. The recrystallization procedure, as described, should remove all traces of color from the commercial preparations (tryptophan may require two crystallizations to accomplish this purpose), and practically all traces of salts, heavy metals, proteins, more soluble compounds and adventitious impurities, and frequently, although not invariably, traces of the racemate from the corresponding optically-active form. The more meticulous investigator may wish to perform two or more recrystallizations of a given amino acid preparation before he is completely satisfied.

N. *Ion Exchange Chromatography*

The widely used chromatographic analysis of amino acids on sulfonated polystyrene resins introduced by Moore and Stein in 1951 is an excellent tool for evaluating the purity of these compounds. This procedure will indicate the presence of *meso*cystine in L-cystine, alloisoleucine in isoleucine, etc. Readers desiring to use this technique are referred to the latest publications by these investigators (7, 8).

References

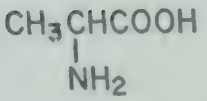
1. Greenstein, J. P., and Winitz, M., *Chemistry of the amino acids*, John Wiley and Sons, New York (in press).
2. Meister, A., *Biochemistry of the amino acids*, Academic Press, New York, 1957.
3. Block, R. J., and Bolling, D., *The amino acid composition of proteins and foods*, 2nd ed., C. C. Thomas, Springfield, Illinois, 1951.
4. Block, R. J., Durrum, E. L., and Zweig, G., *A manual of paper chromatography and paper electrophoresis*, 2nd ed., Academic Press, New York, 1958.
5. Harris, L. J., "A simple process for estimating amino or other basic groups in amino acids, etc.: The 'glacial acetic acid' method," *Biochem. J.*, **29**, 2820 (1935).
6. Sörensen, S. P. L., "Enzymstudien," *Biochem. Z.*, **7**, 45 (1908).
7. Moore, S., Spackman, D. H., and Stein, W. H., "Chromatography of amino acids on sulfonated polystyrene resins. An improved system," *Anal. Chem.*, **30**, 1185 (1958).
8. Spackman, D. H., Stein, W. H., and Moore, S., "Automatic recording apparatus for use in the chromatography of amino acids," *Anal. Chem.*, **30**, 1190 (1958).

Date of issue: June 1960

DL-Alanine

Formula: $C_3H_7O_2N$
Formula Wt.: 89.10
Calc. %: C, 40.44; H, 7.92; N, 15.72; O, 35.92

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.45 Solvent 2 ascending.
R_{BCP} 0.32 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Iminodipropionic acid.

Crystallization Medium: Water, then ethanol to 80% v/v or water:ethanol = 1:4 v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Alanine

Formula: $C_3H_7O_2N$ **Structural Formula:** $CH_3CHCOOH$ with NH_2 on the CH
Formula Wt.: 89.10
Calc. %: C, 40.44; H, 7.92; N, 15.72; O, 35.92.

Source or Method of Preparation: Resolution of synthetic or from natural sources.

Specific Rotation: $+14.7°$ in 1.0 *N* HCl, $c = 5.8$, $t = 15°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.45 Solvent 2 ascending.
R_{BCP} 0.32 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Iminodipropionic acid, vitamin B_6.

Crystallization Medium: Water, then ethanol to 80% v/v or water:ethanol = 1:4 v/v.

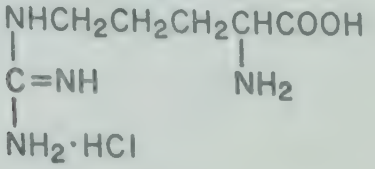
Stability and Storage: Stable.

Date of issue: June 1960

L-Arginine Hydrochloride*

Formula: $C_6H_{14}O_2N_4 \cdot HCl$
Formula Wt.: 210.68
Calc. %: C, 34.21; H, 7.18; N, 26.60; O, 15.19; Cl, 16.83.

Structural Formula:



Source or Method of Preparation: Gelatin, blood meal, seed globulins.

* Also available as free base: Molecular Wt.: 174.21; N = 32.16%.

Specific Rotation: +26.9° in 6 *N* HCl, $c = 2$, $t = 25°$ calculated as free base.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.26 Solvent 2 ascending.
R_{BCP} 0.09 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.5%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Ornithine.

Crystallization Medium: Water pH 5 to 7, then ethanol to 80% v/v.

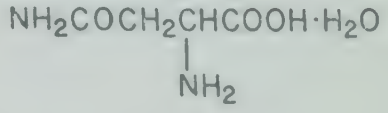
Stability and Storage: Stable if kept dry.

Date of issue: June 1960

L-Asparagine Monohydrate*

Formula: $C_4H_{10}O_4N_2$
Formula Wt.: 150.12
Calc. %: C, 32.00; H, 6.71; N, 18.66; O, 42.63.

Structural Formula:



Source or Method of Preparation: Lupine seedlings.

* Also available as anhydrous asparagine: Molecular Wt. = 132.1; N = 21.21%.

Specific Rotation: $+32.6°$ in 0.1 *N* HCl calc. as asparagine, $c = 1$, $t = 20°$.

Homogeneity: Determined by paper chromatography:
Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.53 Solvent 1 descending.
R_{BCP} 0.11 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Assays: Amide nitrogen (2 *N* HCl, reflux for 2 hours): 9.4%.

Volatile Matter: Not more than 12%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Aspartic acid, tyrosine.

Crystallization Medium: Water, or water and ethanol.

Stability and Storage: Slowly effloresces in dry air.

Date of issue: June 1960

DL-Aspartic Acid

Formula: $C_4H_7O_4N$ **Structural Formula:** $HOOC \cdot CH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 133.11
Calc. %: C, 36.09; H, 5.30; N, 10.52; O, 48.08.

Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.25 Solvent 1 ascending.
R_f 0.22 Solvent 2 descending.
R_{BCP} 0.11 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Aspartic Acid

Formula: $C_4H_7O_4N$ **Structural Formula:** $HOOC \cdot CH_2CH(NH_2)COOH$
Formula Wt.: 133.11
Calc. %: C, 36.09; H, 5.30; N, 10.52; O, 48.08

Source or Method of Preparation: Natural sources or hydrolysis of L-asparagine.

Specific Rotation: $+25.4°$ in 5 *N* HCl, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.25 Solvent 1 ascending.
R_f 0.22 Solvent 1 descending.
R_{BCP} 0.11 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Glutamic acid, NH_4Cl, cystine, asparagine.

Crystallization Medium: Water, then ethanol to 80%, v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Cysteine Hydrochloride Monohydrate*

Formula: $C_3H_9O_3NS \cdot HCl$
Formula Wt.: 175.64
Calc. %: C, 20.51; H, 5.74; N, 7.98; O, 27.33; S, 18.26; Cl, 20.19.

Structural Formula:

$$\begin{array}{l} HSCH_2CHCOOH \cdot H_2O \\ \quad\quad\quad | \\ \quad\quad\quad NH_2 \cdot HCl \end{array}$$

Source or Method of Preparation: Reduction of cystine.

* Also available as anhydrous cysteine: Formula Wt.: = 121.15; N = 11.56%.

Specific Rotation: $+6.53°$ in 5 *N* HCl calc. as cysteine, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2.
Two dimensional: Method A after condensing with N-ethylmaleimide.
Literature Reference: Block, R. J., Durrum, E. L., and Zweig, G., *Paper chromatography and paper electrophoresis*, Academic Press, New York, 1958.
Color Reagent: Ninhydrin.
Specific Reagents: Nitroprusside.
R_f 0.08 Solvent 2 ascending.

Volatile Matter: Cannot be dried by AOAC method without decomposition.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Cystine, tyrosine, H_2S.

Crystallization Medium: Methanol, then ether (peroxide-free).

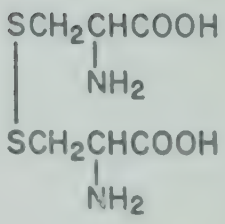
Stability and Storage: Decomposes and oxidizes slowly; hygroscopic.

Date of issue: June 1960

L-Cystine

Formula: $C_6H_{12}O_4N_2S_2$
Formula Wt.: 240.29
Calc. %: C, 29.99; H, 5.03; N, 11.66; O, 26.63; S, 26.68.

Structural Formula:



Source or Method of Preparation: Hair and wool wastes.

Specific Rotation: $-212°$ in 1 *N* HCl, $c = 1$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2.
Color Reagent: Ninhydrin.
Specific Reagents: Nitroprusside—NaCN.
R_f 0.12 Solvent 2 ascending.
R_{BCP} 0.03 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: D-Cystine, *meso*cystine, tyrosine. L-Cystine and *meso*-cystine can be distinguished by infrared spectroscopy (Section J) or by column chromatography (Section N) (lit. ref. 1 below), and separated by recrystallization of the hydrochlorides (lit. ref. 2 below).

Literature References: 1) Wright, N., *J. Biol. Chem.*, **120**, 641 (1937); 2) Loring, H. S., and du Vigneaud, V., *J. Biol. Chem.*, **102**, 287 (1933).

Crystallization Medium: 1.5 *N* HCl, then NH_4OH to neutrality.

Stability and Storage: Stable.

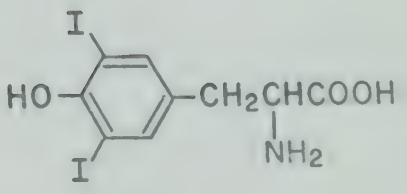
Date of issue: June 1960

3,5-Diiodo-L-tyrosine

Formula: $C_9H_9O_3NI_2$ **Structural Formula:**

Formula Wt.: 433.99

Calc. %: C, 24.96; H, 2.10; N, 3.24; O, 11.09; I, 58.62.



Source or Method of Preparation: Iodination of tyrosine.

Specific Rotation: $+1.5°$ in 1 *N* HCl, $c = 5$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Method C.
Color Reagent: Ninhydrin.
Specific Reagents: $Ce(HSO_4)_4 + NaAsO_2$.
R_f 0.29 Solvent 3b descending.
R_f 0.16 Solvent 3 descending.
R_{BCP} 0.87 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.5%.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Tyrosine, monoiodotyrosine, iodide, iodine.

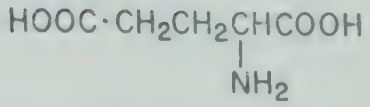
Crystallization Medium: Cold dilute NH_4OH, then acetic acid to pH 6.

Stability and Storage: Decomposes slowly to liberate iodine and iodide.

Date of issue: June 1960

L-Glutamic Acid

Formula: $C_5H_9O_4N$ **Structural Formula:**



Formula Wt.: 147.13

Calc. %: C, 40.81; H, 6.17; N, 9.52; O, 43.50.

Source or Method of Preparation: Wheat gluten, beet sugar filtrate.

Specific Rotation: $+31.5°$ in 5 *N* HCl, $c = 2$, $t = 20°$.

Homogeneity: Determined by paper chromatography.

One dimensional: Solvent 1. Two dimensional: Methods A and B.

Color Reagent: Ninhydrin.

R_f 0.33 Solvent 1 ascending.

R_f 0.33 Solvent 1 descending.

R_{BCP} 0.21 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Aspartic acid, cystine.

Crystallization Medium: Water, then ethanol to 80% v/v.

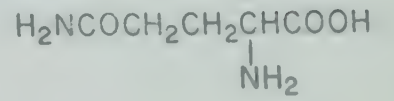
Stability and Storage: Stable.

Date of issue: June 1960

L-Glutamine

Formula: $C_5H_{10}O_3N_2$
Formula Wt.: 146.13
Calc. %: C, 41.09; H, 6.85; N, 19.17; O, 32.824.

Structural Formula:



Source or Method of Preparation: Synthetic or beet extract.

Specific Rotation: +7.0° in water, $c = 2$, $t = 25°$;
+31.8° in 1 *N* HCl, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: None. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.65 Solvent 1 descending.
R_{BCP} 0.13 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Assays: Amide nitrogen (2 *N* HCl, reflux for 2 hrs. or water at 100° for 3 hrs.): 9.6%.

Volatile Matter: Not more than 0.5%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Glutamic acid, NH_4 salt of pyrrolidone carboxylic acid, tyrosine, asparagine, isoglutamine, arginine, Ni.
NOTE: Pyrrolidone carboxylic acid can be visualized on 2-dimensional chromatography by spraying with an acid base indicator.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable; converted to NH_4 salt of pyrrolidone carboxylic acid in hot water.

Date of issue: June 1960

Glycine

Formula: $C_2H_5O_2N$ **Structural Formula:** H_2NCH_2COOH
Formula Wt.: 75.07
Calc. %: C, 32.00; H, 6.72; N, 18.66; O, 42.63.

Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.48 Solvent 1 ascending.
R_f 0.56 Solvent 1 descending.
R_{BCP} 0.15 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.2%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Ammonium salt, iminodiacetic acid and its ammonium salt, nitrilotriacetic acid, NH_4Cl.

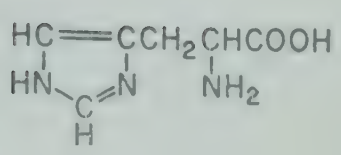
Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Histidine*

Formula: $C_6H_9O_2N_3$ **Structural Formula:**



Formula Wt.: 155.16

Calc. %: C, 46.44; H, 5.85; N, 27.09; O, 20.62.

Source or Method of Preparation: Blood meal, hemoglobin.

* As free base. Also available as the dihydrochloride.

Specific Rotation: $+13.0°$ in 6 *N* HCl, $c = 1$, $t = 25°$.

Homogeneity: Determined by paper chromatography.

One dimensional: Solvent 2. Two dimensional: Methods A and B.

Color Reagent: Ninhydrin.

Specific Reagents: Pauly.

R_f 0.80 Solvent 1 descending.

R_f 0.22 Solvent 2 ascending.

R_{BCP} 0.07 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.5% for histidine·HCl.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Arginine.

Crystallization Medium: Water, then ethanol to 80% v/v.

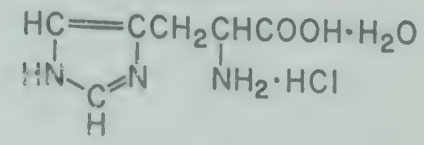
Stability and Storage: Stable.

Date of issue: June 1960

L-Histidine Monohydrochloride Monohydrate

Formula: $C_6H_{12}N_3O_3Cl$
Formula Wt.: 209.6
Calc. %: C, 34.38; H, 5.77; N, 20.05; O, 22.90; Cl, 16.91.

Structural Formula:



Source or Method of Preparation: Blood meal, hemoglobin.

Specific Rotation: +13.0° in 6 *N* HCl calc. as histidine, $c = 1$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Pauly.
R_f 0.80 Solvent 1 descending.
R_f 0.22 Solvent 2 ascending.
R_{BCP} 0.07 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.5% for histidine·HCl.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Arginine.

Crystallization Medium: Water, then ethanol to 80% v/v.

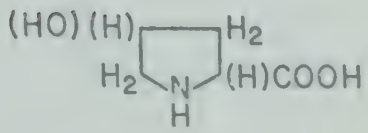
Stability and Storage: Stable.

Date of issue: June 1960

L-Hydroxyproline

Formula: $C_5H_9O_3N$
Formula Wt.: 131.13
Calc. %: C, 45.79; H, 6.92; N, 10.68, O, 36.61.

Structural Formula:



Source or Method of Preparation: Collagen, gelatin.

Specific Rotation: $-76.7°$ in H_2O, $c = 20$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagents: Ninhydrin and isatin.
Specific Reagents: Isatin followed by Ehrlich.
R_f 0.74 Solvent 1 descending.
R_f 0.20 Solvent 2 ascending.
R_{BCP} 0.18 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Assays: Amino group (ninhydrin according to Van Slyke): 0.0%.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Proline.

Crystallization Medium: Ethanol:methanol = 1:1 v/v.

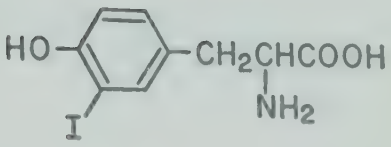
Stability and Storage: Stable.

Date of issue: June 1960

3-Iodo-L-tyrosine

Formula: $C_9H_{10}O_3NI$
Formula Wt.: 307.09
Calc. %: C, 35.19; H, 3.28; N, 4.56; O, 15.62; I, 41.32

Structural Formula:



Source or Method of Preparation: Iodination of tyrosine.

Specific Rotation: $-4.4°$ in 1 *N* HCl, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Method C.
Color Reagent: Ninhydrin.
Specific Reagents: $Ce(HSO_4)_4$—$NaAsO_2$
R_f 0.38 Solvent 3 descending.
R_f 0.59 Solvent 3b descending.
R_{BCP} 0.70 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Tyrosine, diiodotyrosine, iodide, iodine.

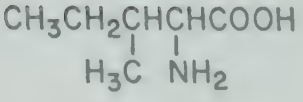
Crystallization Medium: Dilute NH_4OH at room temperature, then acetic acid to pH 6.

Stability and Storage: Keep cold, liberates iodine.

Date of issue: June 1960

L-Isoleucine

Formula: $C_6H_{13}O_2N$ **Structural Formula:**



Formula Wt.: 131.17

Calc. %: C, 54.94; H, 9.99; N, 10.68; O, 24.40.

Source or Method of Preparation: Natural sources.

Specific Rotation: $+40.6°$ in 6.1 *N* HCl, $c = 5.1$, $t = 20°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvents 1 and 3b. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.87 Solvent 1 descending.
R_f 0.82 Solvent 2 ascending.
R_{BCP} 0.76 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol. (This amino acid is poorly separated from leucine.)

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Leucine, tyrosine, methionine, alloisoleucine, D-isoleucine.

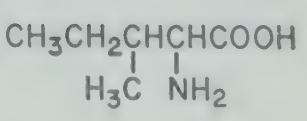
Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Isoleucine + D-Alloisoleucine*

Formula: $C_6H_{13}O_2N$ **Structural Formula:**



Formula Wt.: 131.17

Calc. %: C, 54.94; H, 9.99; N, 10.68; O, 24.40

Source or Method of Preparation: Synthetic.

Specific Rotation: $\pm 3°$ in 5 *N* HCl, $c = 5$, $t = 20°$.

Homogeneity: Determined by paper chromatography.

One dimensional: Solvents 1 and 3b. Two dimensional: Methods A and B.

Color Reagent: Ninhydrin.

R_f 0.87 Solvent 1 descending.

R_f 0.82 Solvent 2 ascending.

R_{BCP} 0.76 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol. (This amino acid is poorly separated from leucine.)

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Leucine, D-isoleucine, L-alloisoleucine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

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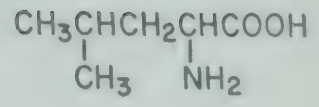
* Some commercial samples of labeled DL-isoleucine are the epimeric mixture of L-isoleucine and D-alloisoleucine. The detection of alloisoleucine is best carried out by infra-red spectrophotometry (Section J of the General Remarks and Analytical Procedures for amino acids), or by column chromatography on ion exchange resins (lit. refs. 7, 8). If a pure sample of L-isoleucine is available, further assays can be done using micro-biological methods.

Date of issue: June 1960

DL-Leucine

Formula: $C_6H_{13}O_2N$ **Structural Formula:** 

Formula Wt.: 131.17

Calc. %: C, 54.94; H, 9.99; N, 10.68; O, 24.40.

Source or Method of Preparation: Synthetic.

Specific Rotation: None (± 1.0 in 5 *N* HCl).

Homogeneity: Determined by paper chromatography.
One dimensional: Solvents 2 and 3B. Two dimensional: Methods A and C.
Color Reagent: Ninhydrin.
R_f 0.88 Solvent 1 descending.
R_f 0.85 Solvent 2 ascending.
R_f 0.82 Solvent 2 descending.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

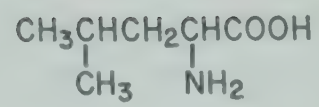
Likely Impurities: L-Isoleucine, D-alloisoleucine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

L-Leucine

Formula: $C_6H_{13}O_2N$ **Structural Formula:** 
Formula Wt.: 131.17
Calc. %: C, 54.94; H, 9.99; N, 10.68; O, 24.40.

Source or Method of Preparation: Casein, wheat gluten, hemoglobin.

Specific Rotation: $+15.6°$ in 5 *N* HCl, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvents 2 and 3b. Two dimensional: Methods A and C.
Color Reagent: Ninhydrin.
R_f 0.88 Solvent 1 descending.
R_f 0.85 Solvent 2 ascending.
R_f 0.82 Solvent 2 descending.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Isoleucine, valine, methionine.

Crystallization Medium: Water, then ethanol to 80% v/v.

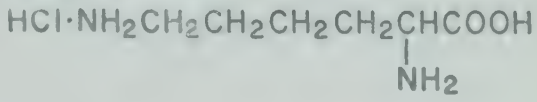
Stability and Storage: Stable.

Date of issue: June 1960

L-Lysine Monohydrochloride*

Formula: $C_6H_{14}O_2N_2 \cdot HCl$
Formula Wt.: 182.7
Calc. %: C, 39.45; H, 8.28; N, 15.34; O, 17.52; Cl, 19.41.

Structural Formula:



The free base contains 19.16% N; Formula Wt. = 146.2.
Source or Method of Preparation: Natural sources or resolution of synthetic.

* Also available as the dihydrochloride.

Specific Rotation: +25.9° in 5 *N* HCl, calc. as free base, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.92 Solvent 1 descending.
R_f 0.18 Solvent 2 ascending.
R_{BCP} 0.05 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.5%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Arginine, D-lysine, diaminopimelic acid, glutamic acid.

Crystallization Medium: For lysine·HCl, water at pH 4 to 6, then ethanol to 80% v/v; for lysine·2 HCl, methanol, then ether, in the presence of excess HCl.

Stability and Storage: Stable at less than 60% relative humidity; above this the dihydrate is formed.

Date of issue: June 1960

DL-Methionine

Formula: $C_5H_{11}O_2NS$ **Structural Formula:** $CH_3SCH_2CH_2CHCOOH$ with NH_2 on the second carbon

Formula Wt.: 149.21

Calc. %: C, 40.25; H, 7.43; N, 9.39; O, 21.45; S, 21.49

Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Platinic iodide.
R_f 0.83 Solvent 1 descending.
R_f 0.50 Solvent 2 ascending.
R_{BCP} 0.53 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Methionine sulfoxide, methionine sulfone, CH_3SH, ethionine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Protect from light and abrasion.

Date of issue: June 1960

L-Methionine

Formula: $C_5H_{11}O_2NS$ **Structural Formula:** $CH_3SCH_2CH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 149.21
Calc. %: C, 40.25; H, 7.43; N, 9.39; O, 21.45; S, 21.49

Source or Method of Preparation: Natural sources or resolution of synthetic.

Specific Rotation: $+21.2°$ in 0.2 *N* HCl, $c = 0.8$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Platinic iodide.
R_f 0.83 Solvent 1 descending.
R_f 0.50 Solvent 2 ascending.
R_{BCP} 0.53 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Methionine sulfoxide, methionine sulfone, CH_3SH, D-methionine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Protect from light and abrasion.

Date of issue: June 1960

DL-Phenylalanine

Formula: $C_9H_{11}O_2N$ **Structural Formula:** $C_6H_5CH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 165.19
Calc. %: C, 65.44; H, 6.71; N, 8.48; O, 19.37

Source or Method of Preparation: Synthetic from benzaldehyde.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Method A.
Color Reagent: Ninhydrin.
R_f 0.90 Solvent 1 descending.
R_f 0.80 Solvent 2 ascending.
R_{BCP} 0.69 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

Part of NAS-NRC Publication 719

L-Phenylalanine

Formula: $C_9H_{11}O_2N$ **Structural Formula:** $C_6H_5CH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 165.19
Calc. %: C, 65.44; H, 6.71; N, 8.48; O, 19.37

Source or Method of Preparation: Resolution of synthetic or from natural sources.

Specific Rotation: $-34.0°$ in H_2O, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Method A.
Color Reagent: Ninhydrin.
R_f 0.90 Solvent 1 descending.
R_f 0.80 Solvent 2 ascending.
R_{BCP} 0.69 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Leucines, valine, methionine, tyrosine, D-phenylalanine.

Crystallization Medium: Water, then ethanol to 80% v/v.

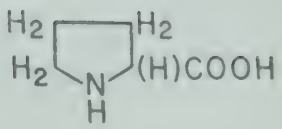
Stability and Storage: Stable.

Date of issue: June 1960

L-Proline

Formula: $C_5H_9O_2N$
Formula Wt.: 115.13
Calc. %: C, 52.16; H, 7.88; N, 12.17; O, 27.79

Structural Formula:



Source or Method of Preparation: Collagen, wheat gluten.

Specific Rotation: $-85.1°$ in H_2O, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Method A.
Color Reagents: Ninhydrin and isatin.
R_f 0.30 Solvent 2 ascending.
R_{BCP} 0.35 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Assays: Amino group (ninhydrin according to Van Slyke) 0.0%.

Volatile Matter: Not more than 0.5%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Hydroxyproline.

Crystallization Medium: Absolute ethanol.

Stability and Storage: Hygroscopic; keep in a desiccator.

Date of issue: June 1960

DL-Serine

Formula: $C_3H_7O_3N$ **Structural Formula:** $HOCH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 105.09
Calc. %: C, 34.28; H, 6.72; N, 13.33; O, 45.67

Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Nessler and HIO_4.
R_f 0.43 Solvent 1 ascending.
R_f 0.47 Solvent 1 descending.
R_{BCP} 0.14 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Dry to constant weight at 105° C. at atmospheric pressure, 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Glycine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Store in desiccator after drying.

Date of issue: June 1960

L-Serine

Formula: $C_3H_7O_3N$ **Structural Formula:** $HOCH_2CHCOOH$ with NH_2 on the CH
Formula Wt.: 105.09
Calc. %: C, 34.28; H, 6.72; N, 13.33; O, 45.67

Source or Method of Preparation: Resolution of synthetic.

Specific Rotation: $+14.5°$ in 1.0 *N* HCl, $c = 9.3$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Nessler and HIO_4.
R_f 0.43 Solvent 1 ascending.
R_f 0.47 Solvent 1 descending.
R_{BCP} 0.14 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Dry to constant weight at 105° C. at atmospheric pressure, 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Glycine, D-serine

Crystallization Medium: Water, then ethanol to 80% v/v.

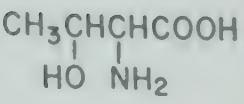
Stability and Storage: Store in a desiccator after drying.

Date of issue: June 1960

DL-Threonine

Formula: $C_4H_9O_3N$
Formula Wt.: 119.12
Calc. %: C, 40.33; H, 7.62; N, 11.76; O, 40.30

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Nessler and HIO_4.
R_f 0.55 Solvent 1 ascending.
R_f 0.61 Solvent 1 descending.
R_{BCP} 0.24 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Dry to constant weight at 105° C. at atmospheric pressure, 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Allothreonine*, glycine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Store in a desiccator after drying.

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* *Cf.* Section J of General Remarks and Analytical Procedures for amino acids.

Date of issue: June 1960

L-Threonine

Formula: $C_4H_9O_3N$ **Structural Formula:** $CH_3CH(OH)CH(NH_2)COOH$
Formula Wt.: 119.12
Calc. %: C, 40.33; H, 7.62; N, 11.76; O, 40.30

Source or Method of Preparation: Resolution of synthetic.

Specific Rotation: $-28.4°$ in water, $c = 1.0$, $t = 26°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 1. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Nessler and HIO_4.
R_f 0.55 Solvent 1 ascending.
R_f 0.61 Solvent 1 descending.
R_{BCP} 0.24 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Dry to constant weight at 105°C. at atmospheric pressure, 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of water has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Allothreonine*, glycine, D-threonine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Store in a desiccator after drying.

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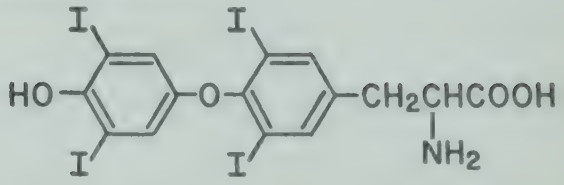
* *Cf.* Section J of General Remarks and Analytical Procedures for amino acids.

Date of issue: June 1960

L-Thyroxine

Formula: $C_{15}H_{11}O_4NI_4$
Formula Wt.: 776.93
Calc. %: C, 23.19; H, 1.43; N, 1.80; O, 8.24; I, 65.35.

Structural Formula:



Source or Method of Preparation: Natural sources or resolution of synthetic.

Specific Rotation: +26° in ethanol-*N* HCl (2:1), c = 1–2, t = 22°.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Method C.
Color Reagent: Ninhydrin.
Specific Reagents: $Ce(HSO_4)_4$—$NaAsO_2$.
R_{BCP} 1.14 Solvent 3b descending.
R_f 0.60 Solvent 3 descending.
R_{BCP} 1.20 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Tyrosine, other iodotyrosines and iodothyronines*, iodide*, iodine*.

Crystallization Medium: Dilute NH_4OH at room temperature, then acetic acid to pH 6.

Stability and Storage: Decomposes slowly in the cold.

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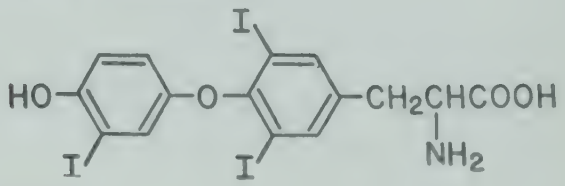
* These impurities are often present in thyroxine-I^{131} and may account for one half of the total radioactivity.

Date of issue: June 1960

3,5,3′-Triiodo-L-thyronine

Formula: $C_{15}H_{12}O_4NI_3$
Formula Wt.: 650.98
Calc. %: C, 27.67; H, 1.85; N, 2.15; O, 9.83; I, 58.49.

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: +21.5° in *N* HCl-ethanol (1:2), $c = 2$, $t = 29.5°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Method C.
Color Reagent: Ninhydrin.
Specific Reagents: $Ce(HSO_4)_4$—$NaAsO_2$.
R_{BCP} 1.46 Solvent 3b descending.
R_{BCP} 1.13 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: *Cf.* thyroxine.

Crystallization Medium: Dilute NH_4OH at room temperature, then acetic acid to pH 6.

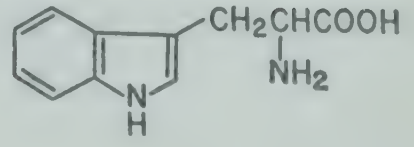
Stability and Storage: Decomposes slowly in the cold.

Date of issue: June 1960

DL-Tryptophan

Formula: $C_{11}H_{12}O_2N_2$
Formula Wt.: 204.22
Calc. %: C, 64.69; H, 5.93; N, 13.72; O, 15.67

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Ehrlich.*
R_f 0.60 Solvent 3 ascending.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Crystallization Medium: 65% v/v ethanol.

Stability and Storage: Store in a dark place; darkens on prolonged exposure to light.

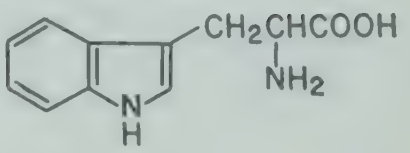
* Impurities in the solvents may interfere with this reaction.

Date of issue: June 1960

L-Tryptophan

Formula: $C_{11}H_{12}O_2N_2$
Formula Wt.: 204.22
Calc. %: C, 64.69; H, 5.93; N, 13.72; O, 15.67

Structural Formula:



Source or Method of Preparation: Resolution of synthetic.

Specific Rotation: $-33.3°$ in H_2O, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 3b. Two dimensional: Solvents A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Ehrlich.*
R_f 0.60 Solvent 3 ascending.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute NaOH has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: D-Tryptophan.

Crystallization Medium: 65% v/v ethanol.

Stability and Storage: Store in a dark place; darkens on prolonged exposure to light.

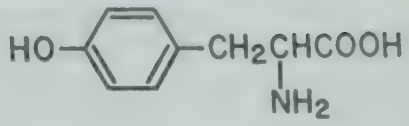
* Impurities in the solvents may interfere with this reaction.

Date of issue: June 1960

DL-Tyrosine

Formula: $C_9H_{11}O_3N$
Formula Wt.: 181.19
Calc. %: C, 59.66; H, 6.12; N, 7.73; O, 26.49

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Pauly.
R_f 0.69 Solvent 1 descending.
R_f 0.60 Solvent 2 ascending.
R_{BCP} 0.41 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Ortho-tyrosine.
Literature Reference: LAMBOOY, J. P., *J. Am. Chem. Soc.*, 78, 771 (1956) on paper chromatograms developed with Solvent 1.

Crystallization Medium: Dilute NH_4OH, then acetic acid to pH 5.

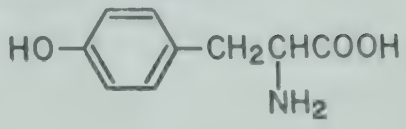
Stability and Storage: Stable.

Date of issue: June 1960

L-Tyrosine

Formula: $C_9H_{11}O_3N$
Formula Wt.: 181.19
Calc. %: C, 59.66; H, 6.12; N, 7.73; O, 26.49

Structural Formula:



Source or Method of Preparation: Casein, hair.

Specific Rotation: $-10.0°$ in 5 N HCl, $c = 2$, $t = 25°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvent 2. Two dimensional. Methods A and B.
Color Reagent: Ninhydrin.
Specific Reagents: Pauly.
R_f 0.69 Solvent 1 descending.
R_f 0.60 Solvent 2 ascending.
R_{BCP} 0.41 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: Ammonium salt, L-cystine.

Crystallization Medium: Dilute NH_4OH, then acetic acid to pH 5.

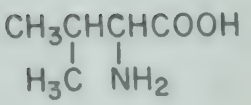
Stability and Storage: Stable.

Date of issue: June 1960

DL-Valine

Formula: $C_5H_{11}O_2N$
Formula Wt.: 117.15
Calc. %: C, 51.26; H, 9.47; N, 11.96; O, 27.32

Structural Formula:



Source or Method of Preparation: Synthetic.

Specific Rotation: None.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvents 2 and 3b. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.81 Solvent 1 descending.
R_{BCP} 0.57 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

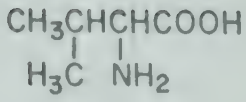
Date of issue: June 1960

Part of NAS-NRC Publication 719

L-Valine

Formula: $C_5H_{11}O_2N$
Formula Wt.: 117.15
Calc. %: C, 51.26; H, 9.47; N, 11.96; O, 27.32

Structural Formula:



Source or Method of Preparation: Resolution of synthetic.

Specific Rotation: $+28.8°$ in 6.0 *N* HCl, $c = 3.4$, $t = 20°$.

Homogeneity: Determined by paper chromatography.
One dimensional: Solvents 2 and 3b. Two dimensional: Methods A and B.
Color Reagent: Ninhydrin.
R_f 0.81 Solvent 1 descending.
R_{BCP} 0.57 Solvent 2 descending. R_{BCP} refers to the distance moved by the amino acid calculated as the fraction of the distance moved by bromcresol purple, which is applied as a 0.1% w/v solution in ethanol.

Volatile Matter: Not more than 0.3%.

Water-insoluble Material: 5 g. in 50 ml. of dilute HCl has a turbidity not greater than given by 0.4 mg. of $BaSO_4$ in 50 ml. of water.

Ash (sulfated): Less than 0.1%.

Heavy Metals (as Pb): Not more than 20 ppm.

Likely Impurities: D-Valine.

Crystallization Medium: Water, then ethanol to 80% v/v.

Stability and Storage: Stable.

Date of issue: June 1960

CARBOHYDRATES AND RELATED SUBSTANCES

General Remarks and Analytical Procedures

The majority of commercially available carbohydrates meet exceedingly high standards of purity, comparable to those for USP dextrose. It is usually safe to assume that acidity and anion content are within or close to the limits set for USP dextrose—hence, specifications or criteria for these items have been omitted. While the melting point of free sugars may be reproducible when determined by one worker with one apparatus, this physical constant is unsatisfactory as a criterion of purity. On the other hand, optical rotatory power is nearly an ideal property for this purpose, particularly when the specific rotation is relatively large. However, even rotatory values may fail to detect small amounts of contaminating carbohydrates and so paper chromatography—a simple and inexpensive qualitative procedure of great delicacy—is here adopted as a routine method for ascertaining homogeneity.

When kept tightly sealed in amber glass containers and stored away from strong light in a cool place, the carbohydrates are stable for an indefinite period of time.

Notes on Analytical Procedures

A. *Specific Rotation:*

Unless otherwise noted, specific rotations are determined in water at 20°C. on samples dried to constant weight *in vacuo* at 60°C. (see Sec. C below) using the D-line of sodium. Concentration is expressed in grams of substance per 100 ml. of solution. For substances which mutarotate, only the equilibrium rotation is measured. One drop of a solution which has been made by diluting ordinary concentrated aqueous ammonia (28%) with an equal volume of water may be added in making up a 25 ml. solution of the sugar in order to hasten mutarotation. The specific rotation, $[\alpha]_D^{20}$, is calculated according to the formula $\alpha \cdot v/l \cdot w$, where α is the observed rotation in circular degrees, v the volume of the solution in ml., l the length of the polarimeter tube in dm. and w the weight of the sample in grams. For hydrated materials the calculation is based on the normal form of the substance, the weight loss on drying being used to adjust the specific rotation to correspond with the given formula weight.

B. *Melting Point:*

With carbohydrate derivatives which are optically inactive, melting points are used as criteria of purity. Any of a large variety of apparatus may be used, the temperature being raised at a rate of from 1° to 2° per minute during the actual determination. The values given are corrected for stem exposure (if any) and represent the range from the first appearance of liquid to the disappearance of the last crystal.

C. *Loss of Weight on Drying:*

Unless otherwise specified under individual sugars, the general procedure for determining volatile contaminants (normally water) is the following one, described in Section 29.6 ("Vacuum Drying—Official") of the *Official Methods of Analysis of the Association of Official Agricultural Chemists*, AOAC, 8th edition, Washington, D. C., 1955, p. 532. "Dry 2–5 g. of a representative sample (powdered, if necessary) in a flat dish (Ni, Pt, or Al) with a tight-fit cover, 2 hours at not over 70° C. (preferably 60° C.), under pressure not exceeding 50 mm. Hg. Remove the dish from oven, cover, cool in desiccator, and weigh. Redry 1 hour and repeat the process until change in weight between successive dryings at 1 hour intervals is not more than 2 mg.

Note: Bleed oven with current of dry air during drying to insure removal of H_2O vapors."

D. *Paper Partition Chromatography; General Procedure*[1]*:*

(1) *Paper.* Whatman No. 1 filter paper in sheets 18¼ x 22½ inches is used, the "grain" of the paper (indicated by an arrow on the package) being in the direction of the longer dimension. Chromatography, whether ascending or descending, is conducted across the grain of the paper, the vertical dimension always being 18¼ inches and the horizontal dimension varying according to the number of samples to be tested or the capacity of the container used. Where descending chromatography is to be used and it is desired to allow the liquid front to run off the paper, the lower end of the paper is serrated with a pair of dressmaker's pinking shears (coarse teeth) in order to obtain uniform run-off.

(2) *Chromatography.* Either ascending or descending chromatography may be used, although the latter is often preferable since the process may be continued beyond the stage when the advancing front reaches the end of the paper. A 100 mg. sample of the carbohydrate to be tested is dissolved in water to make a volume of 1.0 ml. and a 2 μl. (2 lambda) aliquot is applied on a line which is lightly ruled about 6 cm. from, and parallel to, the edge of the paper whence the solvent front will start. The application is made portionwise so that the spot is not more than 3 mm. in diameter. Evaporation may be hastened through the use of a gentle stream of warm air, the ordinary household hair dryer being useful for this purpose. Other spots may be placed on the line at about 4 cm. intervals. The paper containing the materials is exposed for 4 hours at the eventual chromatographic temperature to an atmosphere saturated with the solvent system to be used. The edge of the paper nearest the spots is then dipped into the liquid phase in a suitably enclosed chamber (either a glass cylinder or a chromatography chest which is protected from drafts or other influences that might result in uneven temperatures in the chamber) and kept at a

fairly constant temperature (± 3°) for the length of time specified or until the liquid front has approached to approximately 2.5 cm from the end of the paper. The paper is then dried in a gentle stream of air (hood, hair dryer) and the spots developed.

(3) *Solvent Systems.* (Reagent grade materials are used without further purification).

(a) System 1. 1-Butanol-pyridine-water, 3.0:2.0:1.5 by volume.

(b) System 2. 1-Propanol-water, 8.9:2.8 by volume.

(c) Special solvent systems to be specified under individual sugars.

(4) *Spraying Reagents.*

(a) Aniline hydrogen phthalate reagent[2]: 1.66 g. phthalic acid, 0.93 g. freshly distilled aniline, 100 ml. 1-butanol saturated with water. The reagent is stable for a considerable period of time when stored in a brown glass bottle in the refrigerator; it should be discarded after darkening becomes marked. The reagent is sprayed evenly upon the paper but not in sufficient quantity to run. The paper is then dried at 105–110° C. for 5 to 10 minutes; respraying and redrying intensifies the spots. Weak spots may be verified by viewing the paper under ultraviolet light.*

(b) Periodate-permanganate reagent[3]: 2% (w/v) aqueous $NaIO_4$ and freshly prepared 1% (w/v) $KMnO_4$ in 2% (w/v) aqueous Na_2CO_3. The two solutions are mixed 4:1 by volume just prior to use and sprayed lightly and evenly on the paper. When the spots have appeared, excess reagent is removed by washing the paper in water. Weak spots may be intensified by spraying with a solution made as follows: 1 g. benzidine, 8 g. trichloracetic acid, 20 ml. glacial acetic acid, 12 ml. water and 150 ml. of absolute alcohol.[4]

(c) Ammoniacal silver nitrate reagent[5]: 5 g. $AgNO_3$, 95 ml. water and 6 ml. conc. ammonia. In order to avoid the danger of explosive silver residues, the reagent should be made up just before use and discarded promptly thereafter. The paper is sprayed with the reagent and left overnight in the dark to develop. The background coloring is then removed with "Kodak Liquid X-ray Fixer," the paper washed with water and dried.

E. *Heavy Metals:*

Dissolve 2 g. in 20 ml. of water, add 0.5 ml. of 1 *N* HCl and 10 ml. of a freshly prepared, saturated aqueous solution of hydrogen sulfide. Any darkening produced should not be more than in a blank to which 0.02 mg. of Cu has been added.

F. *Iron:*

Dissolve 1 g. in 10 ml. of water. Add 1 ml. of conc. HCl, about 30 mg. of ammonium persulfate and 15 ml. of butanolic potassium thiocyanate.

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* Radiation of approximately 2540 Å wavelength such as is provided by the model V-41 "Mineralight" of Ultra-Violet Products, Inc., So. Pasadena, Cal., is satisfactory.

Shake vigorously and allow to separate. Any red color in the clear butanol layer is not darker than in a blank to which 0.005 mg. of Fe has been added. *Reagent:* Dissolve 10 g. of KSCN in 10 ml. of H_2O. Warm this solution to 25° to 30° C., add sufficient butanol to make 100 ml. and shake vigorously until clear.

G. *Arsenic:*

The method used is that given under "Dextrose" in *Reagent Chemicals*, American Chemical Society, Washington, D. C., 1955, pp. 151 and 4, and in various textbooks and collections of official methods of analysis. The A.C.S. description is quoted here.

The arsenic in a sample (5 g.) is determined by the Gutzeit method. If the amount of stain produced is less than that given by 0.002 mg. of arsenic, the sample contains less than 0.5 ppm of As.

"The widely used Gutzeit method for arsenic, which is prescribed for testing reagents, depends upon the measurement or comparison of stains produced by the action of evolved arsine on strips of paper which have been impregnated with mercuric bromide from an alcoholic solution. Details of the method are given in collections of official methods of analysis and in textbooks. Because of the nature of the test it is important to have the greatest possible uniformity in the preparation of the stains from samples and from measured amounts of arsenic.

"*Apparatus:* A wide-mouthed bottle of about 60-ml. capacity serves for the generator. It carries a glass tube about 1 cm. in diameter and 6 to 7 cm. long, which is constricted at the bottom to pass through the stopper of the bottle. The tube is to hold glass wool, purified cotton, or similar material, moistened with a 10 per cent solution of lead acetate. All tubes of a set of generators should be charged with equal amounts of this material. The solution serves to hold back any hydrogen sulfide generated in the bottle, and also helps to maintain a uniform content of moisture in the evolved gases.

"Above the lead acetate tube is a narrow glass tube 2.6 to 2.7 mm. in internal diameter and 10 to 12 cm. long which holds the strip of mercuric bromide paper. The diameter of this tube must not be large enough to permit curling of the paper strip. The paper strips are best obtained by the purchase of commercially cut strips which are of uniform width of 2.5 mm. and are generally supplied in a manner which facilitates their preparation for use. The strips are soaked about an hour in a 5 per cent solution of mercuric bromide in alcohol. They are drained and allowed to dry in clean air. It is essential that all strips used for a particular test be sensitized in the same manner and at the same time.

"*Procedure:* Place the sample in the generator bottle with water and about 5 ml. of acid. The acid may be either sulfuric or hydrochloric but in

any set of determinations the kind and the amounts of acid in all the bottles must be the same. If acid is used up in dissolving the sample, the amount must be replaced and the bottles for preparation of standards must contain an amount of arsenic-free salt equal to that resulting from the action of acid on the sample. Add 7.5 ml. of potassium iodide solution (10 grams in 100 ml.) and 4 drops of stannous chloride solution (40 grams of $SnCl_2 \cdot 2H_2O$ dissolved in hydrochloric acid to make 100 ml.). Mix, allow to stand 30 minutes at not less than 25° C., and dilute to 40 ml. Prepare the lead acetate tube, removing excess solution, and insert the paper strip in the small tube. Add to the bottle the required amount of zinc, which may be 10 to 15 grams of stick zinc or 2 to 5 grams of granulated zinc. Insert the stopper carrying the tubes and immerse the generator bottle in a water bath which is maintained at a constant temperature from 20° to 25° C. At the end of 1.5 hours remove the paper strips and compare the stains.

"The character of the stain is affected by variables which should be controlled as closely as possible. Moisture is a factor which is regulated in part by the lead acetate solution on the inert material in the tube next to the generator. A major factor is the rate of evolution of arsine and hydrogen. The kind and concentration of acid in the generator can be regulated fairly easily, but special pains must be taken to have the zinc the same in all generators of a set. Uniformity in a set is much more important than the form of the zinc. Good results may be obtained with pieces of stick zinc, mossy zinc, or granulated zinc. The best concentration of acid may depend on the form of the zinc and the amount used in each generator.

"Attention to all the details is necessary to make certain that stains from equal amounts of arsenic in samples and standards shall be of equal length and appearance. This factor must be emphasized if the practice is followed of making a series of standard stains and using a graph based on the relation between amounts of arsenic and length of stain. A control of average arsenic content should give a stain whose length falls on the graph. A blank will show any significant amount of arsenic in the reagents used."

H. *Special Tests:* Certain special tests are required for individual compounds. Details for these are given on the appropriate specification sheets.

(*References, over*)

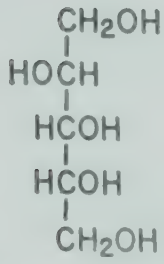
References

1. General references. (a) Lederer, E., and Lederer, M., *Chromatography*, 2nd ed., Elsevier Publishing Co., Amsterdam, 1957. (b) Kowkabany, G. N., "Paper chromatography of carbohydrates and related compounds," *Advances in Carbohydrate Chem.*, **9**, 303 (1954). (c) Jeanes, A., Wise, C. S., and Dimler, R. J., "Improved techniques in paper chromatography of carbohydrates," *Anal. Chem.*, **23**, 415 (1951).
2. Partridge, S. M., "Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars," *Nature*, **164**, 443 (1949).
3. Lemieux, R. U., and Bauer, H. F., "Spray reagent for the detection of carbohydrates," *Anal. Chem.*, **26**, 920 (1954).
4. Wolfrom, M. L., and Miller, J. B., "Detection of carbohydrates on paper chromatograms," *Anal. Chem.*, **28**, 1037 (1956).
5. Dimler, R. J., Schaefer, W. C., Wise, C. S., and Rist, C. E., "Quantitative paper chromatography of D-glucose and its oligosaccharides," *Anal. Chem.*, **24**, 1411 (1952).

Date of issue: June 1960

D-Arabinitol (D-Arabitol)

Formula: $C_5H_{12}O_5$
Formula Wt.: 152.15



Specific Rotation: $[\alpha]_D^{20}$ $+130° \pm 1°$ (*c*, 0.4 in excess acidified molybdate) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

An accurately weighed sample (*ca.* 0.1 g.) is dissolved in 20.0 ml. of stock ammonium molybdate and made up to 25.0 ml. with *N* H_2SO_4.

Stock ammonium molybdate solution: 25.0 g. of the commercial hydrated salt, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (Mallinckrodt analytical grade or the equivalent) is dissolved in distilled water to make 500 ml. of solution and filtered if necessary. Small amounts of crystalline material may separate from this solution on standing; the clear supernatant solution is used for rotatory measurements.

Literature Reference: Richtmyer, N. K., and Hudson, C. S., *J. Am. Chem. Soc.*, **73**, 2249 (1951).

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

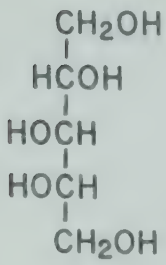
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

L-Arabinitol (L-Arabitol)

Formula: $C_5H_{12}O_5$
Formula Wt.: 152.15



Specific Rotation: $[\alpha]_D^{20}$ $-130° \pm 1°$ (*c*, 0.4 in excess acidified molybdate) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

An accurately weighed sample (*ca.* 0.1 g.) is dissolved in 20.0 ml. of stock ammonium molybdate and made up to 25.0 ml. with *N* H_2SO_4.

Stock ammonium molybdate solution: 25.0 g. of the commercial hydrated salt, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (Mallinckrodt analytical grade or the equivalent) is dissolved in distilled water to make 500 ml. of solution and filtered if necessary. Small amounts of crystalline material may separate from this solution on standing; the clear supernatant solution is used for rotatory measurements.

Literature Reference: Richtmyer, N. K., and Hudson, C. S., *J. Am. Chem. Soc.*, **73**, 2249 (1951).

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

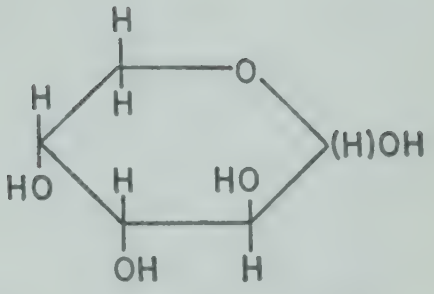
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Arabinose

Formula: $C_5H_{10}O_5$
Formula Wt.: 150.13



Specific Rotation: $[\alpha]_D^{20}$ $-104.5° \pm 0.5°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
Descending chromatography in System 2 for 48 hours or in System 1 shows no contaminants revealed by aniline hydrogen phthalate.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

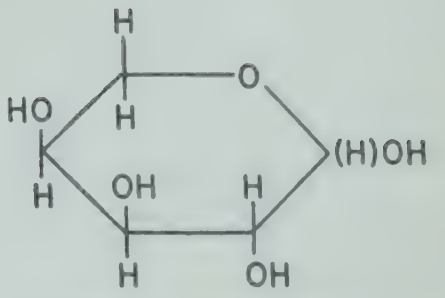
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

L-Arabinose

Formula: $C_5H_{10}O_5$
Formula Wt.: 150.13



Specific Rotation: $[\alpha]_D^{20}$ $+104.5° \pm 0.5°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
Descending chromatography in System 2 for 48 hours or in System 1 shows no contaminants revealed by aniline hydrogen phthalate.

Loss of Weight on Drying: Not more than 0.1%.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

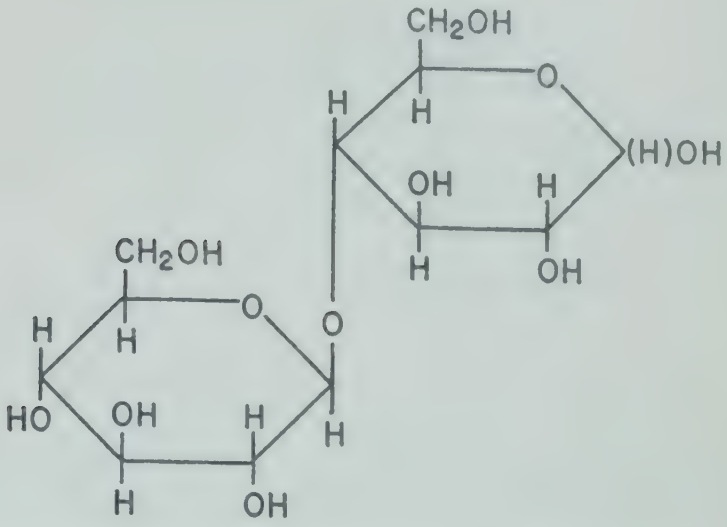
Arsenic (as As): Not more than 0.5 ppm.

NOTE: Standards for L-Arabinose have also been set by ROSIN, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 57.

Date of issue: June 1960

Cellobiose (4-*O*-β-D-Glucopyranosyl-D-glucose)

Formula: $C_{12}H_{22}O_{11}$
Formula Wt.: 342.29



Specific Rotation: $[\alpha]_D^{20}$ +34.6° ± 0.1° (*c*, 8) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: BATES, F. J., AND ASSOCIATES, *Polarimetry, saccharimetry, and the sugars*, U. S. Govt. Printing Office, Washington, D. C., 1942, p. 710.

Homogeneity: Determined by paper chromatography.
Using Systems 1 and 2 (descending chromatography for 48 hours) the one commercial sample tested showed slight contamination with a very slow-moving component (permanganate-periodate spray).

Loss of Weight on Drying: Not over 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

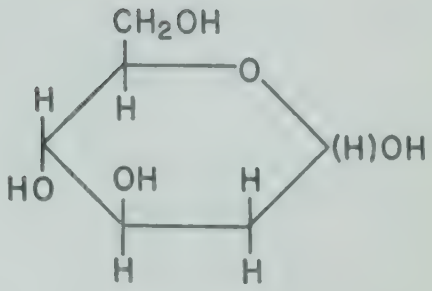
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

2-Deoxy-D-glucose

Formula: $C_6H_{12}O_5$
Formula Wt.: 164.16



Specific Rotation: $[\alpha]_D^{20}$ +46.6° ± 0.2° (*c*, 2) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates. Inspection of the rather extensive literature on 2-deoxy-D-glucose indicates that $[\alpha]_D^{20}$ +46.6° is the most probable value for the rotation of this substance in water.

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate spray after 24 hours chromatography in Systems 1 and 2.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

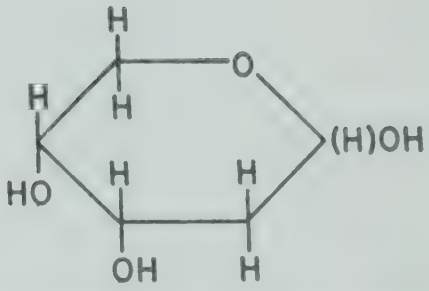
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

2-Deoxy-D-ribose

Formula: $C_5H_{10}O_4$
Formula Wt.: 134.13



Specific Rotation: $[\alpha]_D^{20}$ $-57.3° \pm 0.3°$ (c, 1) determined on sample dried as indicated in section below on Loss of Weight. This is a hitherto unpublished measurement by Mr. Harry W. Diehl, National Institutes of Health.

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate reagent after 24 hours chromatography in Systems 1 and 2.

Loss of Weight on Drying: Not more than 0.1% when dried at 40° and not more than 0.1 mm. for 1.25 hours.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

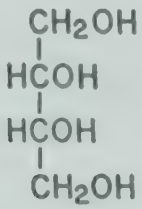
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Erythritol (*meso*-Erythritol, *i*-Erythritol)

Formula: $C_4H_{10}O_4$
Formula Wt.: 122.12



Melting Point: Not less than 118° nor more than 120°.
Parks, G. S., and Anderson, C. T. [*J. Am. Chem. Soc.*, 48, 1506 (1926)] found the freezing point of erythritol to be 118.9°.
Melting points varying from 120° to 126° have been reported for this substance. Erythritol is optically inactive.

Reducing Material: A sample applied to paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

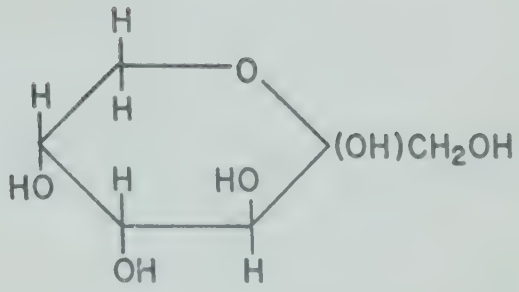
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Fructose (Levulose)

Formula: $C_6H_{12}O_6$
Formula Wt.: 180.16



Specific Rotation: $[\alpha]_D^{20}$ $-92.4° \pm 0.5°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates. Unlike most other sugars, fructose shows a marked change in specific rotation with temperature.

Literature Reference: Isbell, H. S., and Pigman, W. W., *J. Research Natl. Bur. Standards*, **20**, 773 (1938).

Homogeneity: Determined by paper chromatography.
Homogeneous in Systems 1 and 2, sprayed with aniline hydrogen phthalate.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

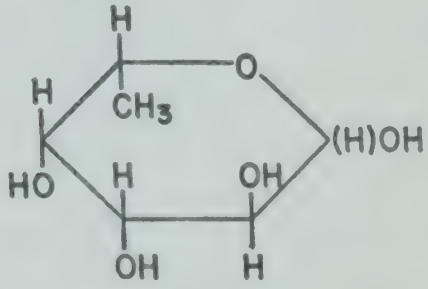
Arsenic (as As): Not more than 0.5 ppm.

NOTE: Standards for D-Fructose have also been set by Rosin, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 241.

Date of issue: June 1960

L-Fucose (6-Deoxy-L-galactose)

Formula: $C_6H_{12}O_5$
Formula Wt.: 164.16



Specific Rotation: $[\alpha]_D^{20}$ $-75.9° \pm 0.2°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: BATES, F. J., AND ASSOCIATES, *Polarimetry, saccharimetry, and the sugars*, U. S. Govt. Printing Office, Washington, 1942, p. 716.

Homogeneity: Determined by paper chromatography.

No contaminants detectable with ammoniacal silver nitrate after paper chromatography in Systems 1 and 2.

NOTE: If not purified through a crystalline derivative, L-fucose may be contaminated with D-mannitol. This impurity, however, is readily detectable on a paper chromatogram with ammoniacal silver nitrate.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

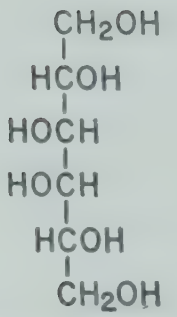
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Galactitol (Dulcitol)

Formula: $C_6H_{14}O_6$
Formula Wt.: 182.17



Melting Point: Not less than 188° nor more than 189°. The melting range of 188 to 189° is most frequently quoted for this substance. Galactitol is optically inactive.

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

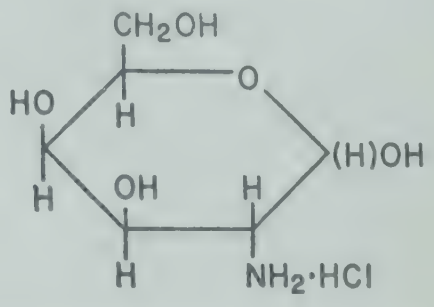
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Galactosamine Hydrochloride (2-Amino-2-deoxy-D-galactose Hydrochloride; Chondrosamine Hydrochloride)

Formula: $C_6H_{13}O_5N \cdot HCl$
Formula Wt.: 215.64



Specific Rotation: $[\alpha]_D^{20}$ $+96.2° \pm 1°$ (c, 1) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates. Based on a measurement reported by GARDELL, S., *Acta Chem. Scand.*, **7**, 207 (1953). Ammonia should not be used to hasten the mutarotation of this substance since it affects the equilibrium value attained.

Homogeneity: Determined by paper chromatography.

Homogeneous when chromatographed in System 2 or in phenol-water and developed with ammoniacal silver nitrate. Two spots (one for the free base and one for the hydrochloride) are observed when the phenol-water system is used.

NOTE: Descending chromatography as described by PARTRIDGE, S. M., *Biochem. J.*, 42, 238 (1948) is used. The trough is filled with phenol saturated with water. In a dish at the bottom of the chromatography chamber is placed a humidifying solution which is made up of water, saturated with phenol, and containing 1% (w/v) NH_3 and a few crystals of KCN. The phenol used in this work should be the best reagent grade available.

Loss of Weight on Drying: Not more than 0.05%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.1%.

Heavy Metals (as Cu): Not more than 10 ppm.

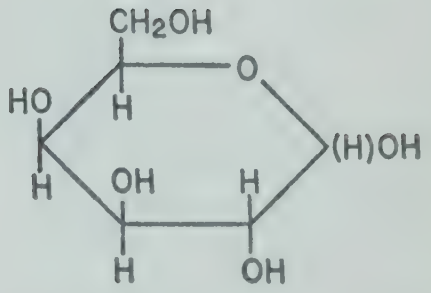
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Galactose

Formula: $C_6H_{12}O_6$
Formula Wt.: 180.16



Specific Rotation: $[\alpha]_D^{20}$ $+80.2° \pm 0.2°$ (c, 5) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.

Descending chromatography for 48 hours in both Systems 1 and 2, followed by aniline hydrogen phthalate spray, reveals, in a typical "C.P." sample, slight contamination with glucose and two other substances which migrate more slowly than galactose. No sample examined has been found to be chromatographically homogeneous.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

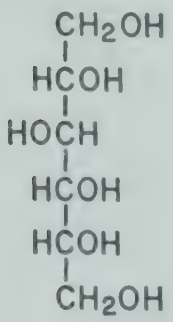
Arsenic (as As): Not more than 0.5 ppm.

NOTE: Standards of purity for D-galactose have also been described by ROSIN, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 202.

Date of issue: June 1960

D-Glucitol (D-Sorbitol, Sorbitol)

Formula: $C_6H_{14}O_6$
Formula Wt.: 182.7



Specific Rotation: $[\alpha]_D^{20}$ $+103° \pm 1°$ (*c*, 0.4 in excess acidified molybdate) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

An accurately weighed sample (*ca.* 0.1 g.) is dissolved in 20.0 ml. of stock ammonium molybdate and made up to 25.0 ml. with *N* H_2SO_4.

Stock ammonium molybdate solution: 25.0 g. of the commercial hydrated salt, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Mallinckrodt analytical grade or the equivalent) is dissolved in distilled water to make 500 ml. of solution and filtered if necessary. Small amounts of crystalline material may separate from this solution on standing; the clear supernatant solution is used for rotatory measurements.

Literature References: Ness, R. K., Fletcher, H. G., Jr., and Hudson, C. S., *J. Am. Chem. Soc.*, **73**, 4759 (1951); Richtmyer, N. K., and Hudson, C. S., *J. Am. Chem. Soc.*, **73**, 2249 (1951).

NOTE: Commercial samples may contain traces of D-mannitol unless they have been purified through the pyridine addition compound.

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

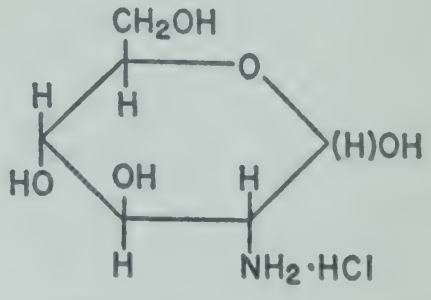
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Glucosamine Hydrochloride (2-Amino-2-deoxy-D-glucose Hydrochloride)

Formula: $C_6H_{13}O_5N \cdot HCl$
Formula Wt.: 215.64



Specific Rotation: $[\alpha]_D^{20}$ $+72.5° \pm 0.7°$ (c, 1) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates. Ammonia should not be used to hasten the mutarotation of this substance since it affects the equilibrium value attained.

Literature Reference: IRVINE, J. C., AND EARL, J. C., *J. Chem. Soc.*, **121**, 2370 (1922).

Homogeneity: Determined by paper chromatography.

Homogeneous when chromatographed in System 2 or in phenol-water and developed with ammoniacal silver nitrate. Two spots (one for the free base and one for the hydrochloride) are observed when the phenol-water system is used.

NOTE: Descending chromatography as described by PARTRIDGE, S. M., *Biochem. J.*, **42**, 238 (1948) is used. The trough is filled with phenol saturated with water. In a dish at the bottom of the chromatography chamber is placed a humidifying solution which is made up of water, saturated with phenol, and containing 1% (w/v) NH_3 and a few crystals of KCN. The phenol used in this work should be the best reagent grade available.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.1%.

Heavy Metals (as Cu): Not more than 10 ppm.

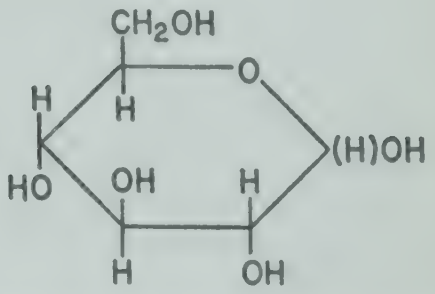
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Glucose, Anhydrous (Dextrose, Anhydrous)

Formula: $C_6H_{12}O_6$
Formula Wt.: 180.16



Established Specifications Adopted: The Committee on Biological Chemistry accepts the specifications for D-glucose established by the American Chemical Society with the additional specification (given below) based on paper chromatography.

Literature Reference: THE A. C. S. COMMITTEE ON ANALYTICAL REAGENTS, *Reagent chemicals*, American Chemical Society, Washington, D. C., 1955, p. 150.

Homogeneity: Determined by paper chromatography.
Homogeneous in both Systems 1 and 2 (17 hours, descending chromatography, aniline hydrogen phthalate spray).

NOTES: The purification of D-glucose has been studied extensively and samples of certified analysis have for some years been available from the National Bureau of Standards.

Literature Reference: BATES, F. J., AND ASSOCIATES, *Polarimetry, saccharimetry, and the sugars*, U. S. Govt. Printing Office, Washington, D. C., 1942, pp. 390, 551.

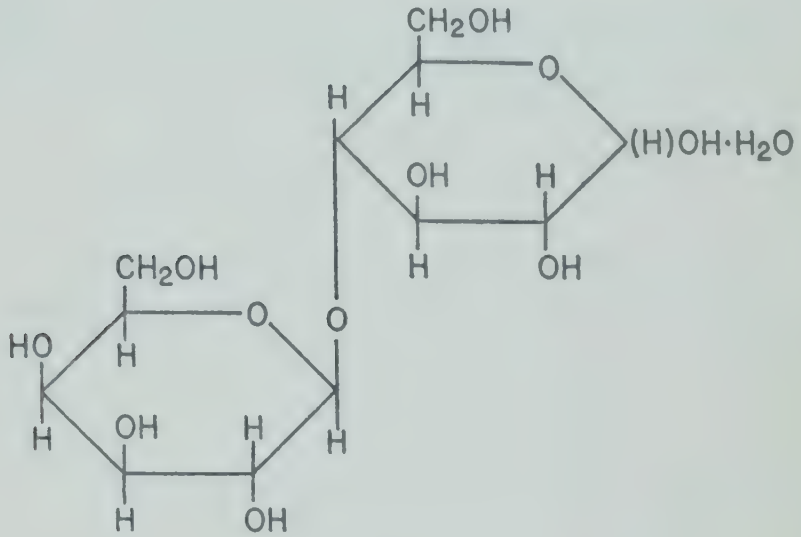
Standards for D-glucose have also been set by: a) ROSIN, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 159; b) *The Pharmacopeia of the United States*, 15th revision (U. S. P. XV), Mack Publishing Co., Easton, Pa., 1955, p. 202.

Date of issue: June 1960

Part of NAS-NRC Publication 719

Lactose Monohydrate (4-*O*-β-D-Galactopyranosyl-D-glucose Monohydrate)

Formula: $C_{12}H_{22}O_{11} \cdot H_2O$
Formula Wt.: 360.31



Specific Rotation: $[\alpha]_D^{20}$ $+52.6° \pm 0.5°$ (*c*, 8) determined on sample dried at 80° for two hours.

Literature References: See *The Pharmacopeia of the United States*, 15th revision (U. S. P. XV), Mack Publishing Co., Easton, Pa., 1955, p. 373; ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.

No contaminants detectable with ammoniacal silver nitrate after 48 hours of descending chromatography in either System 1 or 2. Because of the relatively low solubility of lactose in water only ½ the standard quantity of material is applied to the paper.

Loss of Weight on Drying: Not more than 0.1% after drying at 80° for 2 hrs.

Water-insoluble Material: Should give clear, colorless solution in water.

Dextrins: A solution of 0.5 g. in 10 ml. of water shows no coloration when treated with a few drops of dilute iodine solution.

(*cont. over*)

Date of issue: June 1960

C-17 (cont.)

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

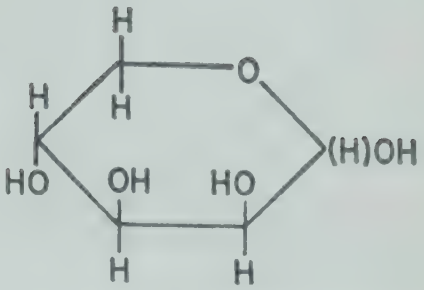
Arsenic (as As): Not more than 0.5 ppm.

NOTE: Standards for lactose have been described in *The Pharmacopeia of the United States*, 15th revision (U. S. P. XV), Mack Publishing Co., Easton, Pa., 1955, p. 373, and in ROSIN, J., *Reagent chemicals and standards*, D. Van Nostrand Co., New York, 1955, p. 229.

Date of issue: June 1960

D-Lyxose

Formula: $C_5H_{10}O_5$
Formula Wt.: 150.13



Specific Rotation: $[\alpha]_D^{20}$ $-13.8° \pm 0.4°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate after chromatography in Systems 1 and 2.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

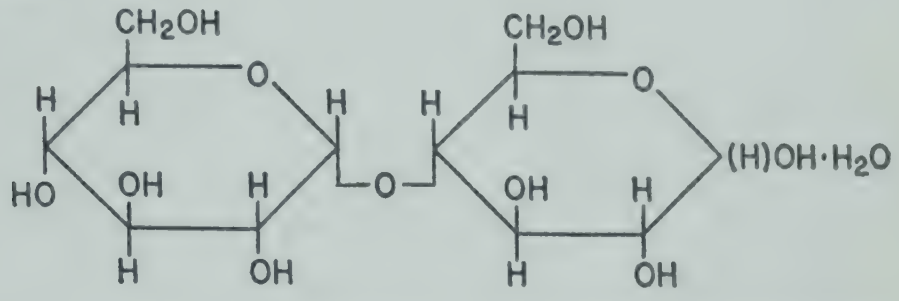
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Maltose Monohydrate (4-*O*-α-D-Glucopyranosyl-D-glucose Monohydrate)

Formula: $C_{12}H_{22}O_{11} \cdot H_2O$
Formula Wt.: 360.31



Specific Rotation: $[\alpha]_D^{20}$ +130.4° ± 1.3° (*c*, 4) determined on undried sample and calculated on monohydrate basis.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.

No commercial samples of maltose have been found to be homogeneous on paper chromatography in Systems 1 and 2.

Loss of Weight on Drying to constant weight at 100° and not more than 0.5 mm. pressure: Not more than 6%.

NOTE: Owing to drying methods used, commercial maltose may contain less water of crystallization than the 5.00% theoretically required for the monohydrate. This moisture is lost only slowly at 60° *in vacuo* but relatively rapidly at 100° and 0.5 mm. It should be noted that the anhydrous material is hygroscopic; therefore weighings ought to be made in a closed container.

Literature Reference: For a study of the dehydration of maltose, see CLELAND, J. E., AND FETZER, W. R., *Ind. Eng. Chem., Anal. Ed.*, **14**, 27 (1942).

Water-insoluble Material: Should give clear, colorless solution in water.

Dextrins: A solution of 0.5 g. in 10 ml. of water should give no coloration when treated with several drops of dilute iodine solution.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

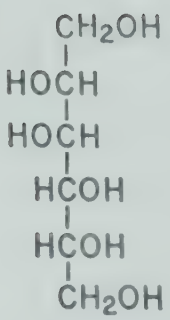
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Mannitol

Formula: $C_6H_{14}O_6$
Formula Wt.: 182.17



Specific Rotation: $[\alpha]_D^{20}$ $+141° \pm 1°$ (*c*, 0.4 in excess acidified molybdate) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

An accurately weighed sample (*ca.* 0.1 g.) is dissolved in 20.0 ml. of stock ammonium molybdate and made up to 25.0 ml. with *N* H_2SO_4.

Stock ammonium molybdate solution: 25.0 g. of the commercial hydrated salt, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (Mallinckrodt analytical grade or the equivalent) is dissolved in distilled water to make 500 ml. of solution and filtered if necessary. Small amounts of crystalline material may separate from this solution on standing; the clear supernatant solution is used for rotatory measurements.

Literature Reference: RICHTMYER, N. K., AND HUDSON, C. S., *J. Am. Chem. Soc.*, **73**, 2249 (1951).

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

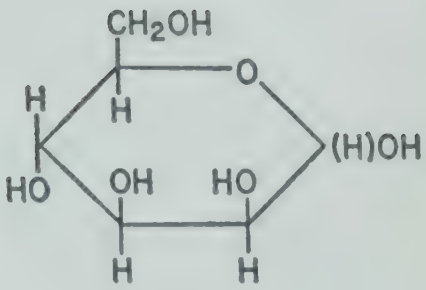
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Mannose

Formula: $C_6H_{12}O_6$
Formula Wt.: 180.16



Specific Rotation: $[\alpha]_D^{20}$ $+14.2° \pm 0.4°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate after chromatography in both Systems 1 and 2.

Loss of Weight on Drying: Not over 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

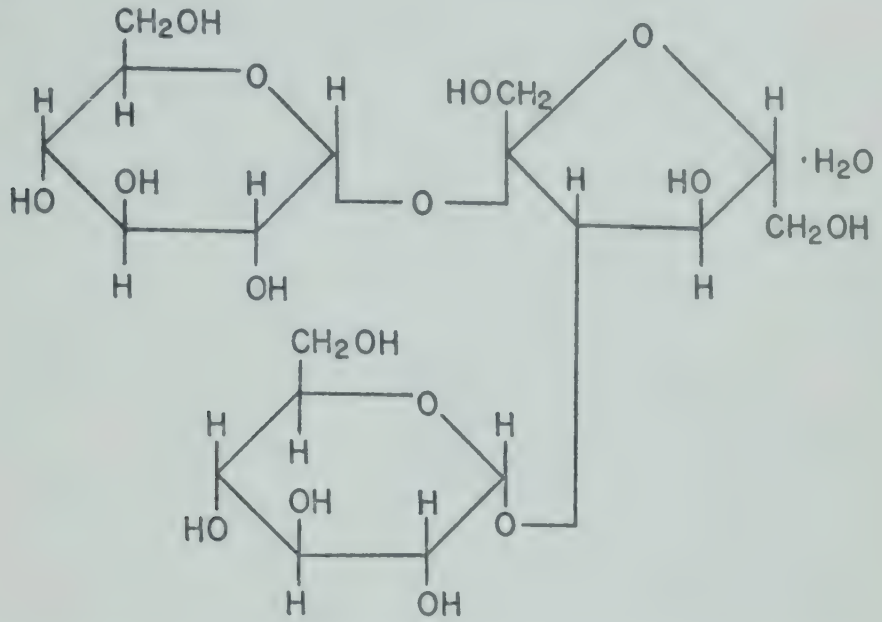
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Melezitose Monohydrate* (*O*-α-D-Glucopyranosyl-(1 → 2)-*O*-β-D-fructofuranosyl-(3 → 1) α-D-Glucopyranoside Monohydrate)

Formula: $C_{18}H_{32}O_{16} \cdot H_2O$
Formula Wt.: 522.46



Specific Rotation: $[\alpha]_D^{20}$ +91.7° ± 0.5° (*c*, 3) determined on sample dried as in section below on Loss of Weight and calculated on the basis of monohydrate.

Literature Reference: RICHTMYER, N. K., AND HUDSON, C. S., *J. Org. Chem.*, **11**, 610 (1946).

Homogeneity: Determined by paper chromatography.

No contaminants detectable by periodate-permanganate reagent after 48 hours chromatography in both Systems 1 and 2.

Loss of Weight on Drying at 110° and *ca.* 1 mm. pressure: Not over 3.6%.

These conditions were employed by Richtmyer and Hudson, cited above. A value of 3.45% corresponds to the theoretical amount expected of a monohydrate.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

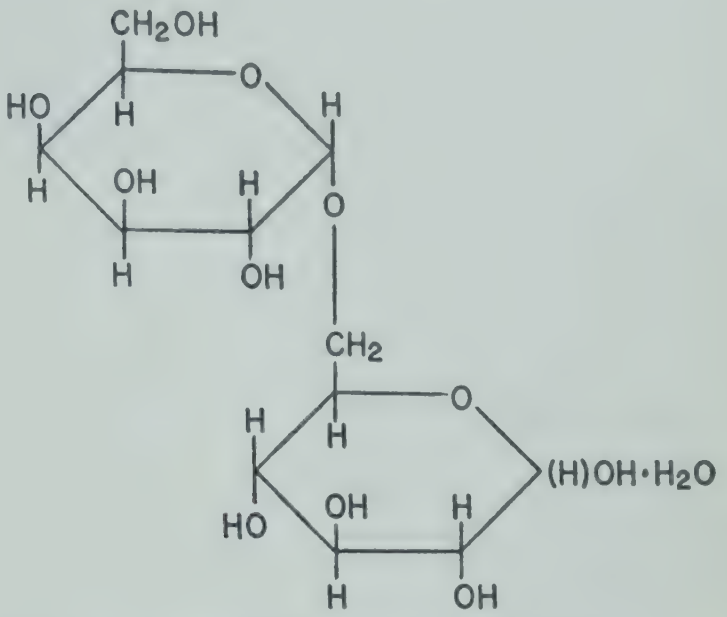
Arsenic (as As): Not more than 0.5 ppm.

* Richtmyer and Hudson, cited above, have shown that the long-known dihydrate of melezitose effloresces on standing to a monohydrate and that anhydrous melezitose deliquesces in the air to afford the same monohydrate. They concluded, therefore, that the monohydrate "appears to be the stable form of the sugar under normal atmospheric conditions" and recommended the use of this form for the preparation of melezitose solutions of accurate composition. Melezitose monohydrate is normally obtained when the sugar is crystallized from aqueous alcohol.

Date of issue: June 1960

Melibiose Monohydrate* (6-*O*-α-D-Galactopyranosyl-D-glucose Monohydrate)

Formula: $C_{12}H_{22}O_{11} \cdot H_2O$
Formula Wt.: 360.31



Specific Rotation: $[\alpha]_D^{20}$ +135.2° ± 0.7° (*c*, 4) determined on sample dried as in section below on Loss of Weight and calculated on the monohydrate basis. Fletcher and Diehl found $[\alpha]_D^{20}$ +142.3° (anhydrous basis). The value presented is calculated from theirs to the monohydrate basis.

Literature Reference: FLETCHER, H. G., JR., AND DIEHL, H. W., *J. Am. Soc.*, **74**, 5774 (1952).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by ammoniacal silver nitrate after descending chromatography for 48 hours in both Systems 1 and 2.

Loss of Weight on Drying: Not more than 5.1% when dried to constant weight at 100° and not more than 0.5 mm.

NOTE: The α-anomer may be crystallized from aqueous alcohol as the monohydrate or from dry methanol in essentially anhydrous form. On standing, the anhydrous modification absorbs moisture from the atmosphere and approaches the monohydrate in water content. The figure quoted is based on the 5.0% of water theoretically contained in the monohydrate plus the 0.1% tolerance for additional moisture normally acceptable in the case of unhydrated sugars.

(*cont. over*)

* For many years melibiose was known only as the dihydrate of the β-anomer. In 1952 Fletcher and Diehl (cited above) investigated a new form of this sugar and found it to be the monohydrate of the α-anomer. The superior crystallizing qualities and lower solubility of the α-monohydrate make it the preferable form in which to isolate this sugar. For this reason it seems likely that α-melibiose monohydrate will, in the future, be the predominant commercial form of this sugar.

Date of issue: June 1960

C-23 (cont.)

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

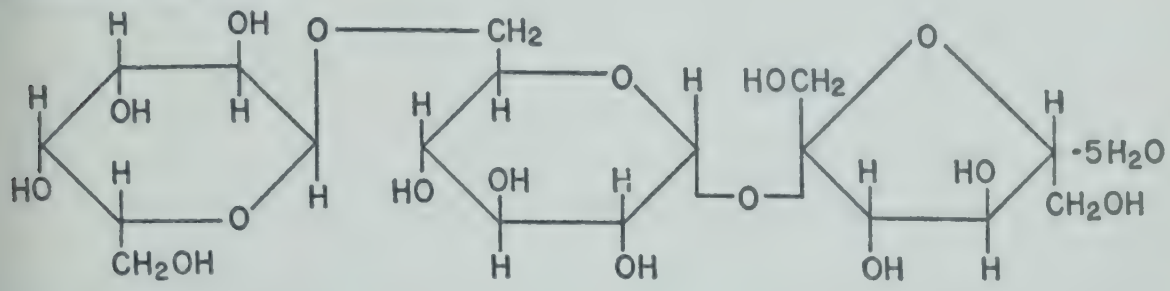
Arsenic (as As): Not more than 0.5%.

Date of issue: June 1960

Part of NAS-NRC Publication 719

Raffinose Pentahydrate (*O*-α-D-Galactopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl β-D-Fructofuranoside Pentahydrate)

Formula: $C_{18}H_{32}O_{16} \cdot 5H_2O$
Formula Wt.: 594.52



Specific Rotation: $[\alpha]_D^{20}$ +105.2° ± 0.7° (*c*, 4) determined on sample dried as in section below on Loss of Weight and calculated on pentahydrate basis.

Literature Reference: BATES, F. J., AND ASSOCIATES, *Polarimetry, saccharimetry, and the sugars*, U. S. Govt. Printing Office, Washington, D. C., 1942, p. 750.

Homogeneity: Determined by paper chromatography.
No contaminants detectable with periodate-permanganate spray after 48 hours chromatography in Systems 1 and 2.

Loss of Weight on Drying to constant weight at 78° and *ca.* 0.5 mm. pressure: Not more than 15.3%. Theory for the pentahydrate is 15.15%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

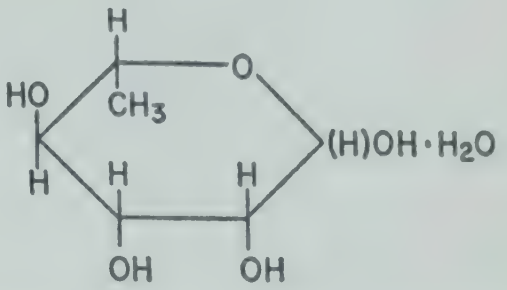
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

L-Rhamnose Monohydrate (6-Deoxy-L-mannose Monohydrate)

Formula: $C_6H_{12}O_5 \cdot H_2O$
Formula Wt.: 182.17



Specific Rotation: $[\alpha]_D^{20}$ $+8.2° \pm 0.4°$ (c, 4) determined on sample dried as described in section below on Loss of Weight.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate after chromatography in Systems 1 and 2.

Loss of Weight on Drying to constant weight at 34° and 0.5 mm. pressure: Not more than 0.1%.

NOTE: Attempts to remove water of crystallization from this hydrate at 64° *in vacuo* results in partial melting, the loss of water being less than theory. Under the conditions specified here water of crystallization does not appear to be lost.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

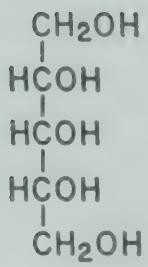
Iron (as Fe): Not more than 5 ppm.

Arsenic: Not more than 0.5 ppm.

Date of issue: June 1960

Ribitol (Adonitol)

Formula: $C_5H_{12}O_5$
Formula Wt.: 152.15



Melting Point: Not less than 101° nor more than 102°. The melting point of 102° which FISCHER, E., *Ber.*, **26,** 633 (1893) reported for ribitol has been confirmed by various more recent authors. This glycitol is optically inactive.

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

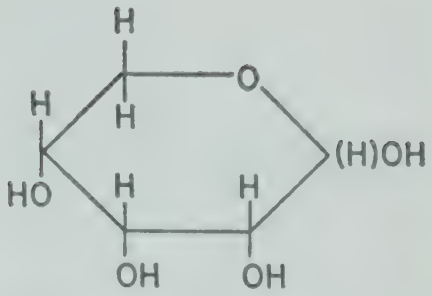
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Ribose

Formula: $C_5H_{10}O_5$
Formula Wt.: 150.13



Specific Rotation: $[\alpha]_D^{20}$ $-20.4° \pm 0.4°$ (c, 2) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates. The value quoted represents a hitherto unpublished measurement by Mr. H. W. Diehl of the National Institutes of Health.

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate after chromatography in both Systems 1 and 2.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

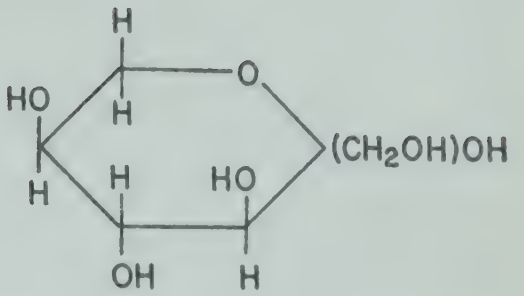
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

L-Sorbose

Formula: $C_6H_{12}O_6$
Formula Wt.: 180.16



Specific Rotation: $[\alpha]_D^{20}$ $-43.4° \pm 0.2°$ (c, 12) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: PIGMAN, W. W., AND ISBELL, H. S., *J. Research Natl. Bur. Standards*, **19**, 443 (1937).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by ammoniacal silver nitrate after chromatography in both Systems 1 and 2.

Loss of Weight on Drying: Not over 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

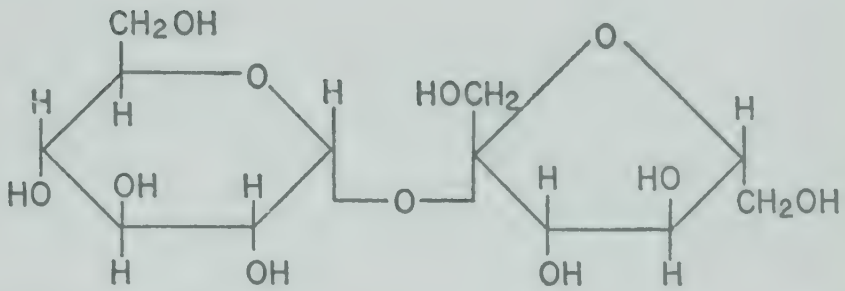
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Sucrose (α-D-Glucopyranosyl β-D-Fructofuranoside)

Formula: $C_{12}H_{22}O_{11}$
Formula Wt.: 342.30



Established Standards Adopted: The Committee on Biological Chemistry adopts the specification for pure sucrose described by the International Commission for Uniform Methods of Sugar Analysis with the additional criteria, given below, based on paper chromatography.

Literature Reference: Supplement to *The International Sugar J.*, June-July (1950), p. 31.

Specific Rotation: $[\alpha]^{20}_{5892.5}$ $+66.529°$ (c, 26).
$[\alpha]^{20}_{5461}$ $+78.342°$ (c, 26).

Reducing Substances (estimated as invert sugar): Not more than 0.004%.

Ash: Not more than 0.004%.

Moisture: Not more than 0.002%.

Homogeneity: Determined by paper chromatography.
Homogeneous in both Systems 1 and 2 (17 hours, descending chromatography, aniline hydrogen phthalate spray).

NOTES: The purification of sucrose has been the object of exhaustive study and for many years specially purified sucrose of certified analysis has been available from the National Bureau of Standards.

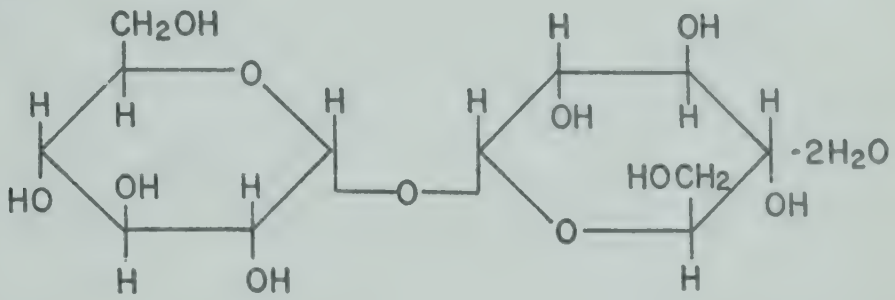
Literature Reference: BATES, F. J., AND ASSOCIATES, *Polarimetry, saccharimetry, and the sugars*, U. S. Govt. Printing Office, Washington, D. C., 1942, pp. 392, 551.

Standards for sucrose have also been set by ROSIN, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 453, and by *The Pharmacopeia of the United States*, 15th revision (U. S. P. XV), Mack Publishing Co., Easton, Pa., 1955, p. 683.

Date of issue: June 1960

Trehalose Dihydrate (α-D-Glucopyranosyl α-D-Glucopyranoside Dihydrate)

Formula: $C_{12}H_{22}O_{11} \cdot 2H_2O$
Formula Wt.: 378.33



Specific Rotation: $[\alpha]_D^{25}$ + 179.9° ± 0.4° (*c*, 7) determined on sample dried as described in section below on Loss of Weight and calculated on dihydrate basis. This specific rotation is the average of three determinations made with a highly purified sample provided by Dr. N. K. Richtmyer, National Institutes of Health.

Homogeneity: Determined by paper chromatography.
No contaminants detectable by periodate-permanganate spray after 48 hours descending chromatography in both Systems 1 and 2.

Loss of Weight on Drying at 60° and not more than 0.5 mm. pressure: Not more than 9.6%. Theoretical moisture content for the dihydrate is 9.52%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

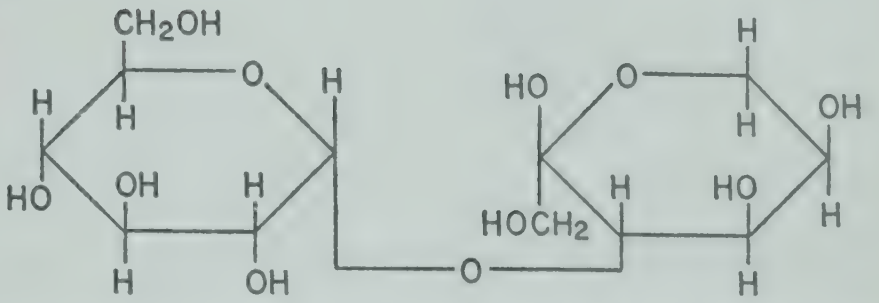
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Turanose (3-*O*-α-D-Glucopyranosyl-D-fructose)

Formula: $C_{12}H_{22}O_{11}$
Formula Wt.: 342.30



Specific Rotation: $[\alpha]_D^{20}$ +75.8° ± 0.4° (*c*, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur. Standards*, **20**, 773 (1938).

Homogeneity: Determined by paper chromatography.

Ammoniacal silver nitrate fails to reveal contaminants after chromatography in both Systems 1 and 2.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

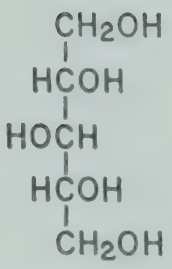
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

Xylitol

Formula: $C_5H_{12}O_5$
Formula Wt.: 152.15



Melting Point: Not less than 93° and not more than 95°. The compound is optically inactive.

NOTE: Xylitol was first crystallized in a hygroscopic, metastable form melting at 61–61.5° (lit. ref. 1 below). This substance was later obtained in a stable modification melting at 93–94.5° (lit. ref. 2 below).

Literature References: 1) Wolfrom, M. L., and Kohn, E. J., *J. Am. Chem. Soc.*, **64**, 1739 (1942); 2) Carson, J. F., Waisbrot, S. W., and Jones, F. T., *J. Am. Chem. Soc.*, **65**, 1777 (1943).

Reducing Material: A sample applied to filter paper as in the standard procedure for paper chromatography gives no coloration with aniline hydrogen phthalate spray.

Loss of Weight on Drying: Not more than 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

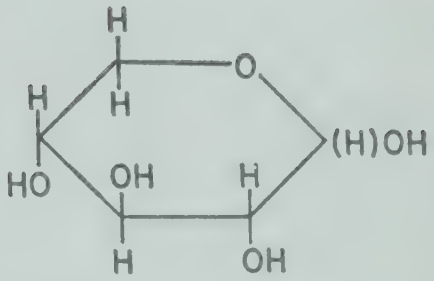
Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

Date of issue: June 1960

D-Xylose

Formula: $C_5H_{10}O_5$
Formula Wt.: 150.13



Specific Rotation: $[\alpha]_D^{20}$ $+18.8° \pm 0.6°$ (c, 4) determined on sample dried as described in Section C of the General Remarks and Analytical Procedures for carbohydrates.

Literature Reference: ISBELL, H. S., AND PIGMAN, W. W., *J. Research Natl. Bur Standards*, **18**, 141 (1937).

Homogeneity: Determined by paper chromatography.
No contaminants detectable by aniline hydrogen phthalate after chromatography in both Systems 1 and 2.

Loss of Weight on Drying: Not over 0.1%.

Water-insoluble Material: Should give clear, colorless solution in water.

Ash: Not more than 0.05%.

Heavy Metals (as Cu): Not more than 10 ppm.

Iron (as Fe): Not more than 5 ppm.

Arsenic (as As): Not more than 0.5 ppm.

NOTE: Standards for D-xylose have been set by ROSIN, J., *Reagent chemicals and standards*, 3rd ed., D. Van Nostrand Co., New York, 1955, p. 482.

Date of issue: June 1960

COENZYMES AND RELATED COMPOUNDS

General Remarks

Since pure preparations of the coenzymes commonly employed in biochemical investigations are not generally available commercially, determination of their purity must of necessity rest on such criteria as enzyme assays. It is essential that the manufacturer provide adequate information concerning the assay conditions used for his preparations. The Criteria sheets are representative of material of the highest available purity. However, less pure preparations may be available and these may be satisfactory so long as they are adequately described and major impurities are indicated.

For the nucleotide coenzymes, where ultraviolet absorption is high, the format of the Criteria sheets is similar to that of the purine and pyrimidine derivatives (*cf.* pp. *Pi–Piv*). The a_M values are the best obtainable, in the judgment of the Committee on Biological Chemistry and its Subcommittee on Coenzymes, but it is recognized that some of these values are not known with certainty.

Date of issue: June 1960

3-Acetylpyridine-*DPN (* = Analog of DPN)

Formula: $C_{22}H_{28}O_{14}N_6P_2$ **Formula Wt.:** 662

Usually available as free acid.

Enzyme Assay:

Suggested Method: Reduction to 3-acetylpyridine-*DPNH using yeast or liver alcohol dehydrogenases.

Literature References: Siegel, J. M., Montgomery, G. A., and Bock, R. M., *Arch. Biochem. Biophys.*, 82, 288 (1959); Kaplan, N. O., and Ciotti, M. M., *J. Biol. Chem.*, 221, 823 (1956).

Experimental Measurement: Increase in absorbancy at 363 mμ ($^{\Delta D}363$). When the assay is linked to another coenzyme (viz., DPN, TPN, etc.), see the appropriate NRC Criteria sheet for the necessary reference constants.

Theory Value per mg.: Theory μMoles/mg. is 1.51.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): 3-acetylpyridine-*DPN (oxidized form) 16.5×10^3 at pH 7.5 and 260 mμ.

Ratio A 250/260 = 0.81, at pH 7.5
A 280/260 = 0.24

Literature Reference: Siegel, J. M., *et al.*, *ibid.*

a_M (Molar Absorbancy): 3-acetylpyridine-*DPNH (reduced form) 9.1×10^3 at pH 10 and 363 mμ.

a_M (Molar Absorbancy): 3-acetylpyridine-*DPN (CN) 8.7×10^3 at pH 10 and 343 mμ.

Literature References: Siegel, J. M., *et al.*, *ibid.*; Kaplan, N. O., and Ciotti, M. M., *ibid.*

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determinations of a_M reference values.

Homogeneity: Evidence for chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

Date of issue: June 1960

Coenzyme A

Formula: $C_{21}H_{36}O_{16}N_7P_3S$ **Formula Wt.:** 767

Usually available as lyophilized free acid in reduced form, CoA-SH.

Enzyme Assay:

Suggested Method: Sulphanilamide acetylation.*

Literature References: KAPLAN, N. O., AND LIPMANN, F., *J. Biol. Chem.*, **174**, 37 (1948); GREGORY, J., NOVELLI, G., AND LIPMANN, F., *J. Am. Chem. Soc.*, **74**, 854 (1952). (Latter gives theory Lipmann Units per mg. for pure CoA-SH; see also below.)

Experimental Measurement: Colorimetric determination of unacetylated sulphanilamide.

When the assay is linked to another coenzyme (viz., DPN, TPN, etc.), see the appropriate NRC Criteria sheet for the necessary reference constants.

Theory Values per mg.: a) Theory μMoles/mg. is 1.31.

b) When "Enzyme Units" employed, theory is 413 Lipmann Units/mg.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): Co-ASH 14.6×10^3, at pH 2 and 260 mμ

Ratio A 250/260 = 0.84, at pH 2

A 280/260 = 0.22

Literature Reference: For spectral constants of AMP employed for characteristic U. V. absorption of CoA see BOCK, R. M., LING, N.-S., MORELL, S. A., AND LIPTON, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

Homogeneity: Evidence of chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

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* This assay measures not only intact CoA-SH, but also the disulphide form. The assay will also measure dephospho-CoA and phosphopantetheine.

Date of issue: June 1960

Part of NAS-NRC Publication 719

Diphosphopyridine Nucleotide (DPN, Coenzyme-I)

Formula: $C_{21}H_{27}O_{14}N_7P_2$ **Formula Wt.:** 663

Usually available as free acid.

Enzyme Assay.

Suggested Method: Reduction to DPNH using yeast alcohol dehydrogenase.

Literature Reference: Ciotti, M. M., and Kaplan, N. O., in S. P. Colowick and N. O. Kaplan (Editors), *Methods of enzymology, Vol. III*, Academic Press, New York, 1957, p. 890.

Experimental Measurement: Increase in absorbancy at 340 mμ ($\Delta D340$). When the assay is linked to another coenzyme (viz., TPN, etc.), see the appropriate NRC Criteria sheet for the necessary reference constants.

Theory Value per mg.: Theory μMoles/mg. is 1.51.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): DPN (oxidized form) 18.0×10^3, at pH 7 and 260 mμ.

Ratio A 250/260 = 0.83, at pH 7
A 280/260 = 0.22

Literature References: Kornberg, A., and Pricer, W. E., in E. E. Snell (Editor), *Biochemical preparations, Vol. 3*, p. 20, J. Wiley and Son, New York, 1953; Siegel, J. M., Montgomery, G. A., and Bock, R. M., *Arch. Biochem. Biophys.*, **82**, 288 (1959).

a_M (Molar Absorbancy): DPN (reduced form) 6.2×10^3, at pH 10 and 340 mμ

a_M (Molar Absorbancy): DPN (CN) 5.9×10^3, at pH 10 and 327 mμ

Ratio A 250/260 = 0.82, at pH 10

Literature References: Horecker, B. L., and Kornberg, A., *J. Biol. Chem.*, **175**, 385 (1948); Siegel, J. M., *et al.*, *ibid.*; Colowick, S. P., Kaplan, N. O., and Ciotti, M. M., *J. Biol. Chem.*, **191**, 447 (1951).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determinations of a_M reference values.

Homogeneity: Evidence for chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

Date of issue: June 1960

Reduced Diphosphopyridine Nucleotide (DPNH)

Formula: $C_{21}H_{29}O_{14}N_7P_2$ **Formula Wt.:** 665

Usually available as sodium salt.

Enzyme Assay:

Suggested Method: Oxidation to DPN using alcohol dehydrogenase and acetaldehyde.

Literature Reference: CIOTTI, M. M., AND KAPLAN, N. O., in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in enzymology, Vol. III*, Academic Press, New York, 1957, p. 893.

Experimental Measurement: Decrease in absorbancy at 340 mμ ($^{\Delta D}340$). When the assay is linked to another coenzyme (viz., DPN, TPN, etc.), see the appropriate NRC Criteria sheet for the necessary reference constants.

Theory Value per mg.: Theory μMoles/mg. is 1.50.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): DPNH 6.2×10^3, at pH 10 and 340 mμ.

Ratio A 250/260 = 0.82, at pH 10

A 280/260 = 0.23

Literature References: HORECKER, B. L., AND KORNBERG, A., *J. Biol. Chem.*, **175**, 385 (1948); SIEGEL, J. M., MONTGOMERY, G. A., AND BOCK, R. M., *Arch. Biochem. Biophys.*, **82**, 288 (1959).

a_M (Molar Absorbancy): DPNH 14.4×10^3, at pH 10 and 259 mμ.

Literature Reference: SIEGEL, J. M., *et al.*, *ibid.*

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determinations of a_M reference values.

Homogeneity: Evidence for chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

Date of issue: June 1960

Triphosphopyridine Nucleotide (TPN, Coenzyme-II)

Formula: $C_{21}H_{28}O_{17}N_7P_3$ **Formula Wt.:** 743

Usually available as sodium salt.

Enzyme Assay:

Suggested Method: Reduction to TPNH by isocitric dehydrogenase.

Literature References: Ciotti, M. M., and Kaplan, N. O., in S. P. Colowick and N. O. Kaplan (Editors), *Methods of enzymology, Vol. III*, Academic Press, New York, 1957, p. 892.

Theory Value per mg.: Theory μMoles/mg. is 1.35.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): TPN (oxidized form) 18.0×10^3, at pH 7 and 260 mμ.

Ratio A 250/260 = 0.83, at pH 7
A 280/260 = 0.21

Literature References: 1) For a_M—Kornberg, A., and Pricer, W. E., in E. E. Snell (Editor), *Biochemical preparations, Vol. 3*, J. Wiley and Son, New York, 1953, p. 28; 2) For ratios—Circular OR-10, *U. V. spectra of 5'-ribonucleotides*, Pabst Laboratories, 1956, p. 19.

a_M (Molar Absorbancy): TPN (reduced form) 6.2×10^3, at pH 10 and 340 mμ

a_M (Molar Absorbancy): TPN (CN) 5.9×10^3, at pH 10 and 327 mμ

Literature References: 1) For a_M—Horecker, B. L., and Kornberg, A., *J. Biol. Chem.*, **175**, 385 (1948); 2) For ratios—Circular OR-10, *ibid.*; Ciotti, M. M., and Kaplan, N. O., *ibid.*

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

Homogeneity: Evidence for chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

Date of issue: June 1960

Uridine Diphosphoglucose (UDPG)

Formula: $C_{15}H_{24}O_{17}N_2P_2$ **Formula Wt.:** 566

Usually available as sodium salt.

Enzyme Assay:

Suggested Method: Oxidation to UDP-glucuronic acid using UDPG dehydrogenase and DPN. (Theory requires 2 moles DPN per mole UDPG).

Literature Reference: Strominger, J. L., Maxwell, E. S., Axelrod, J., and Kalckar, H. M., *J. Biol. Chem.*, **224**, 79 (1957).

Experimental Measurement: Increase in absorbancy at 340 mμ ($\Delta D340$). When the assay is linked to another coenzyme (viz., DPN, TPN, etc.), see the appropriate NRC Criteria sheet for the necessary reference constants.

Theory value per mg.: Theory μMoles/mg. is 1.77.

Spectral Reference Values (Tentative):

a_M (Molar Absorbancy): UDPG 10.8×10^3, at pH 7 and 262 mμ.

Ratio A 250/260 = 0.75, at pH 7

A 280/260 = 0.38

Literature References: The average constants for UMP, UDP and UTP are employed. See: Ploeser, J. M., and Loring, H. S., *J. Biol. Chem.*, **178**, 431 (1949); Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determinations of a_M reference values.

Homogeneity: Evidence for chromatographic homogeneity should be presented, whenever possible, from a minimum of two solvent systems that will permit the detection of small amounts of structurally-related compounds.

Date of issue: June 1960

LIPIDS

General Remarks and Analytical Procedures

The fatty acids and their methyl esters listed in this first edition are prepared by purification of natural products, using the appropriate sequence of physical and chemical techniques. It is felt that the quality of natural fatty acids and their derivatives is indicated by their source and by the methods used in their preparation as well as by the analyses of the products. In the Specifications and Criteria sheets these methods are listed in the order in which they are carried out. In general, they include the following: saponification, removal of unsaponifiable matter by extraction, alcoholysis, esterification, salt separation[1], urea adduct crystallization[2], solvent crystallization[1], fractional distillation[1], hydrogenation[1], and bromination-debromination.[1]

Unless otherwise indicated, the substances may be assumed to give acid, iodine, and saponification values within one per cent of the theoretical values when these analyses are carried out according to official methods of the American Oil Chemists' Society.[3]

For paper chromatography, the method of choice is that of H. Schlenk *et al.*[4] which uses siliconized paper, acetic acid as solvent, and iodine vapor with or without cyclodextrin as means of detection of spots.

The content of conjugated and unconjugated polyenoic acids may be determined by the method of Herb and Riemenschneider.[5] For some acids and esters it is desirable to have data on the content of *trans* acids. These can be obtained from infrared absorption as described by D. Swern *et al.*[6] In general, if the apparatus is available, all types of impurities may be detected and identified most conveniently through gas-liquid chromatography[7] and also by paper chromatography of the hydrogenated products according to the method of Schlenk *et al.*[4] Other general methods of value in characterizing fatty acids and their derivatives are given in Nos. 8 to 12 of the References.

The user of fatty acids and their esters should be aware that many commercially available samples of these substances are grossly impure and some are not the substances represented on the labels. Therefore, it is up to the user to check the purity of products purchased. It is a safe rule to distrust any preparation of unsaturated acid or ester which is not received sealed in an ampoule under inert gas or vacuum.

Unsaturated fatty acids and esters are subject to autoxidation, and even a preparation of high quality must be handled and stored in the absence of oxygen if it is not to deteriorate. Therefore, after each use samples should be replaced under nitrogen or carbon dioxide, or be resealed in vacuum. Storage at low temperatures reduces the danger of oxidation.

References

1. Hilditch, T. P., "*The chemical constitution of natural fats*," John Wiley and Sons, Inc., New York, 1956, p. 570.
2. Schlenk, H., "Urea inclusion compounds of fatty acids," in *Progress in the chemistry of fats and other lipids, Vol. 2*, Pergamon Press, London and New York, 1954, p. 243.
3. *Official and tentative methods of the American Oil Chemists' Society* (35 E. Wacker Drive, Chicago 1, Ill.).
4. Schlenk, H., Gellerman, J. L., Tillotson, J. A., and Mangold, H. K., "Paper chromatography of lipids," *J. Am. Oil Chem. Soc.*, **34**, 377 (1957).
5. Herb, S. F., and Riemenschneider, R. W., "Influence of alkali concentration and other factors on the conjugation of natural polyunsaturated acids as determined by ultraviolet absorption measurements," *J. Am. Oil Chem. Soc.*, **29**, 456 (1952).
6. Swern, D., Knight, H. B., Shrene, O. D., and Heether, M. R., "Comparison of infrared spectrophotometric and lead salt-alcohol methods for determination of *trans* octadecanoic acids and esters," *J. Am. Oil Chem. Soc.*, **27**, 17 (1950).
7. James, A. T., and Martin, A. J. P., "Gas-liquid chromatography: the separation and identification of the methyl esters of saturated and unsaturated acids from formic to *n*-octadecanoic acid," *Biochem. J.*, **63**, 144 (1956).
8. Wheeler, D. H., "Infrared absorption spectroscopy in fats and oils," in *Progress in the chemistry of fats and other lipids, Vol. 2*, Pergamon Press, London and New York, 1954, p. 268.
9. Mangold, H. K., "Chromatography of lipids," in *Progress in the chemistry of fats and other lipids, Vol. 6*, Pergamon Press, London and New York, 1959.
10. Mehlenbacher, V. H., "Standard methods in the fat and oil industry," in *Progress in the chemistry of fats and other lipids, Vol. 5*, Pergamon Press, London and New York, 1958, p. 1.
11. Holman, R. T., "Measurement of polyunsaturated fatty acids," in Glick, D., (Editor), *Methods of biochemical analysis, Vol. 4*, Interscience, New York, 1957, p. 99.
12. Pitt, G. A. J., and Morton, R. A., "Ultraviolet spectrophotometry of fatty acids," in *Progress in the chemistry of fats and other lipids, Vol. 4*, Pergamon Press, London and New York, 1957, p. 228.

Date of issue: June 1960

Arachidonic Acid

Formula: $C_{20}H_{32}O_2$ **Formula Wt.:** 304.5

Structural Formula:

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$$

Source: Hog liver

Method of Preparation:

Petroleum ether extraction of liver
Saponification
Low temperature solvent crystallization
Urea crystallization
Esterification
Fractional distillation
Saponification

Chemical and Physical Properties:

Iodine value: Approx. 327

Total conjugated acids: Approx. 1.0%, as determined by ultraviolet spectrophotometry (Ref. 5).

Homogeneity: One component at room temperature, as determined by paper chromatography. Hydrogenated acid gives one component.

Minimum content of arachidonic acid: 90%

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

L-2

Linoleic Acid

Formula: $C_{18}H_{32}O_2$ **Formula Wt.:** 280.4

Structural Formula:

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$$

Source: Safflower oil

Method of Preparation:

Saponification
Urea crystallization
Low temperature solvent crystallization
Esterification
Fractional distillation
Saponification

Chemical and Physical Properties:

Total conjugated acids: 0.10%, as determined by ultraviolet spectrophotometry (Ref. 5).
Linolenic acid content: 0.05%

Minimum content of linoleic acid: 98%

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Linolenic Acid

Formula: $C_{18}H_{30}O_2$ **Formula Wt.:** 278.4

Structural Formula:

$$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$$

Source: Linseed oil

Method of Preparation:

Saponification
Bromination
Three crystallizations of hexabromides from dioxane
Debromination
Fractional distillation
Saponification
Simple distillation

Chemical and Physical Properties:

Iodine value: 274
Negative Beilstein test
Moles per cent *trans* double bonds: 13, as determined by infrared spectrophotometry (Ref. 6).

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Methyl Arachidonate

Formula: $C_{21}H_{34}O_2$ **Formula Wt.:** 318.5

Structural Formula:

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOCH_3$$

Source: Hog liver

Method of Preparation:

Petroleum ether extraction of liver
Saponification
Low temperature crystallization from solvents
Urea crystallization
Esterification
Fractional distillation

Chemical and Physical Properties:

Iodine value: 300–305
Total conjugated acids: 1.0%, as determined by ultraviolet spectrophotometry (Ref. 5).
Homogeneity: One component, as determined by paper chromatography. Hydrogenated methyl arachidonate gives one component.

Minimum content of methyl arachidonate: 90%

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Methyl Linoleate

Formula: $C_{19}H_{34}O_2$ **Formula Wt.:** 294.5

Structural Formula:

$$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOCH_3$$

Source: Safflower oil

Method of Preparation:

Saponification
Urea crystallization
Solvent crystallization
Esterification
Fractional distillation

Chemical and Physical Properties:

Iodine value: 172.6
Total conjugated acids: 0.10%, as determined by ultraviolet spectrophotometry (Ref. 5).
Linolenic acid content: 0.5%
Homogeneity: One component, as determined by paper chromatography.

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Methyl Linolenate

Formula: $C_{19}H_{32}O_2$ **Formula Wt.:** 292.5

Structural Formula:

$$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOCH_3$$

Source: Linseed oil

Method of Preparation:

Saponification
Bromination
Three crystallizations of hexabromides from dioxane
Debromination
Fractional distillation

Chemical and Physical Properties:

Iodine value: 259.7
Negative Beilstein test
Total conjugated acids: Less than 0.15%, as determined by ultraviolet spectrophotometry (Ref. 5).
Moles per cent *trans* double bonds: 13, as determined by infrared spectrophotometry (Ref. 6).

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Methyl Oleate

Formula: $C_{19}H_{36}O_2$ **Formula Wt.:** 296.5

Structural Formula:

$CH_3(CH_2)_7CH=CH(CH_2)_7COOCH_3$

Source: Olive oil

Method of Preparation:

Interesterification
Fractional distillation
Low temperature crystallization from solvents
Fractional distillation

Chemical and Physical Properties:

Iodine value: 86
Total conjugated acids: 0.02%, as determined by ultraviolet spectrophotometry (Ref. 5).
Linoleic acid: 0.03%
Linolenic acid: nil
Homogeneity: One component, as determined by paper chromatography.

Minimum content of methyl oleate: 99%

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Methyl Palmitate

Formula: $C_{17}H_{34}O_2$ **Formula Wt.:** 270.5

Structural Formula:

$CH_3(CH_2)_{14}COOCH_3$

Source: Crude palmitic acid

Method of Preparation:

Salt separation
Esterification
Fractional distillation
Solvent crystallization

Chemical and Physical Properties:

Iodine value: Less than 0.5
Melting Point: 30° C.
Homogeneity: One component, as determined by paper chromatography.

Minimum content of methyl palmitate: 99%

Storage: Room temperature

Date of issue: June 1960

Methyl Stearate

Formula: $C_{19}H_{38}O_2$ **Formula Wt.:** 298.5

Structural Formula:

$CH_3(CH_2)_{16}COOCH_3$

Source: Crude stearic acid

Method of Preparation:

Salt separation
Esterification
Fractional distillation
Solvent crystallization

Chemical and Physical Properties:

Iodine value: Less than 0.5
Melting point: 38° C.
Homogeneity: One component, as determined by paper chromatography.

Minimum content of methyl stearate: 99%

Storage: Room temperature

Date of issue: June 1960

Oleic Acid

Formula: $C_{18}H_{34}O_2$ **Formula Wt.:** 282.5

Structural Formula:

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

Source: Olive oil

Method of Preparation:

Interesterification
Fractional distillation
Low temperature crystallization from solvents
Fractional distillation
Saponification
Simple distillation

Chemical and Physical Properties:

Iodine value: 89
Total conjugated acids: 0.02%, as determined by ultraviolet spectrophotometry (Ref. 5).
Linoleic acid: 0.03%
Linolenic acid: nil
Homogeneity: One component, as determined by paper chromatography.

Minimum content of oleic acid: 99%.

Storage: Below 5° C. under vacuum or inert gas is recommended.

Date of issue: June 1960

Palmitic Acid

Formula: $C_{16}H_{32}O_2$ **Formula Wt.:** 256.4

Structural Formula:

$CH_3(CH_2)_{14}COOH$

Source: Crude palmitic acid

Method of Purification:

Salt separation
Esterification
Fractional distillation
Solvent crystallization
Saponification

Chemical and Physical Properties:

Melting point: 63.5° C.
Homogeneity: One component, as determined by paper chromatography.

Minimum content of palmitic acid: 99%

Storage: Room temperature

Date of issue: June 1960

Stearic Acid

Formula: $C_{18}H_{36}O_2$ **Formula Wt.:** 284.5

Structural Formula:

$CH_3(CH_2)_{16}COOH$

Source: Crude stearic acid

Method of Purification:

Salt separation
Esterification
Fractional distillation
Solvent crystallization
Saponification

Chemical and Physical Properties:

Iodine value: Less than 0.5
Melting point: 69.6° C.
Homogeneity: One component, as determined by paper chromatography.

Minimum content of stearic acid: 99%

Storage: Room temperature

Date of issue: June 1960

PURINE AND PYRIMIDINE DERIVATIVES

General Remarks and Analytical Procedures

These specifications and criteria may be considered to be "descriptions" in that the Committee on Biological Chemistry has been assured that samples which are essentially pure by these criteria can be obtained commercially. Unless the manufacturer specifies to the contrary in his description, his product may be suspected of containing materials mentioned herein as "more probable impurities."

Careful technique is essential for effective use of these criteria, and some of the more important procedural precautions are discussed in the following notes.

A. *Ultraviolet Absorption Spectra as Criteria of Purity and Character:*

Care is required in the use of quantitative absorption at specific wavelengths as a measure of the amount and purity of a purine or pyrimidine derivative in a given sample. Calibration of the wavelength scale, cleanliness of the quartz cuvettes, and matching the latter at the wavelengths to be used, are of prime importance. Provided that these precautions are taken and the suggested buffer systems are used, the a_M values observed in the different laboratories should agree within two to three per cent. Replicate results in the same laboratory should agree within one to two per cent.

The ratios of optical density at various wavelengths are reasonably sensitive criteria for detecting the presence of contaminants with different spectra. *When* a sample is satisfactory in this respect, the absorption *at the maximum*, where the rate of change of absorption with wavelength is minimal, can then be used as a quantitative measure of the amount of a named compound in a given weight of sample.

Comparison of an experimentally-determined molar absorbancy, a_M, with a reference a_M has been selected as the principal method for establishing the quantity of a named compound which is present. Since a purine or pyrimidine derivative may be available in a variety of forms, such as different salts and hydrates, the Subcommittee on Purines, Pyrimidines and Related Compounds has selected the anhydrous free base or acid as the reference compound that is the subject of each Specifications and Criteria sheet. The supplier may use either this named compound or a specified derivative thereof to describe his product. If a derivative of the named compound is used, its molecular weight or formula weight must be cited by the supplier in order to show the basis for the calculation of the experimentally-observed a_M value (see equation 2 of Section B below). Supporting evidence for the formulation of a particular derivative (elemental analysis, moisture, loss of weight on drying, etc.) should be provided by the supplier insofar as possible.

As is stated on the Specifications and Criteria sheets, reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee invites constant suggestions for revisions, particularly with reference to precise determination of a_M values.

B. *Use of Reference Values:*

Where acceptable spectral ratios establish the identity of a product, absorbancy measurements may be used to determine composition, as shown below. Molar absorbancy (a_M) is defined by the following equation from National Bureau of Standards Circular LC-857 (1947):

Equation 1: $$a_M = \frac{A}{\text{moles per liter}} = \frac{(A)\ (\text{Mol. Wt.})}{\text{gms. per liter}}$$

where A is the optical density observed under the conditions specified.

To determine the percentage of the product which is accounted for by the named compound, the following equation is used:

Equation 2: $$\text{Per cent} = \frac{(100)(\text{Mol. Wt.})(A)}{(a_M)(\text{gms. per liter})}$$

where Mol. Wt. is that of the named compound of the criteria sheet (or the Formula Weight, F. W., of the supplier's product) and a_M is the Reference Value.

C. *Paper Chromatography:*

Chromatographic techniques have become widely used in connection with purine and pyrimidine chemistry, and individual laboratories tend to establish preferences in methodology which may render comparisons among laboratories difficult. Careful control of the following variables is therefore recommended:

(1.) *Solvent System.* Two or more solvent systems should be used, selected for most effective separation from the named compound of the most probable impurities. The purity of the components of the solvent system should be as specified in the literature reference given. If no purity is indicated, the recommended procedure is to use the purest grade obtainable.

(2.) *Chromatographic Technique.* R_f values will vary somewhat with the type of paper used, and the temperature at which the assay is run. With the same solvent, R_f's given for ascending chromatography tend, on the whole, to be somewhat lower than those for descending. The amount of material applied and the size of the spot influence sensitivity considerably. In general, a single application of 200 micrograms will readily permit detection of ultraviolet-absorbing impurities of one per cent. Since the size of the spot affects resolution, it is recommended that the diameter not exceed 0.5 to 1 cm. With compounds of low solubility, it is preferable to

superimpose applications until a total of 200 micrograms has been applied in the prescribed area. Each successive application should be thoroughly dried. Solubility can be improved for some compounds by using dilute HCl or alkali as solvent. (If the spots on the chromatograms are cut out, extracted, and the extracts read in a spectrophotometer, total recovery frequently may not exceed 90 to 95 per cent of the absorbancy in the original spot.)

(3.) *Method of Inspection.* Visualization of the developed chromatogram is most commonly carried out for purine and pyrimidine compounds by direct inspection in a dark room with ultraviolet light. Other methods, such as formation of mercury salts, are acceptable, provided the method used is adequately specified. The ultraviolet light should have a major radiation component which includes the band 250 to 270 mμ. Distilled rather than deionized water is preferred, since resin fines may leak through deionizing columns and appear as ultraviolet absorbing spots near the origin of chromatograms. The quality of the light source used for inspection influences the sharpness with which spots can be visualized and the sensitivity with which trace impurities can be detected. Both lamps and filters may become less efficient with age.

D. *Other Information:*

Biochemical intermediates of high quality should be physically clean (free of lint or foreign particles) and yield brilliant and substantially colorless solutions. Those compounds which can be obtained readily in crystalline form should be identifiable by microscopic examination. Manufacturers are encouraged to provide elemental analyses, or other specific analytical data, wherever this information contributes significantly to confirmation of purity. Some commercial preparations are known for their freedom from trace metals, particularly heavy metals, which is important to the investigator, and presentation of this type of data by the manufacturer is desirable.

(References, over)

Selected References on General Techniques

E. Chargaff and J. N. Davidson (Editors), *The nucleic acids, Vol. I*, Academic P New York, 1955. Chapters on: "Separation of nucleic acid components by chr tography on filter paper" by Wyatt, G. R., pp. 243–64; "Chemistry of purines pyrimidines" by Bendich, A., pp. 81–136; "Optical properties of nucleic acids and t components" by Beaven, G. H., Holiday, E. R., and Johnson, E. A., pp. 493–545

S. P. Colowick and N. O. Kaplan (Editors), *Methods of enzymology, Vol. III*, Acade Press, New York, 1957, pp. 62, 111, 166. See especially "Paper chromatography mononucleotides & oligonucleotides" by Markham, Roy, pp. 743–47; "Methods characterization of nucleic acids by base composition" by Bendich, A., pp. 715–23.

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Block, R. J., *Paper chromatography. A laboratory manual*, Academic Press, New Yo 1955.

"Chromatography," *British Medical Bulletin*, Vol. 10, No. 3 (1954).

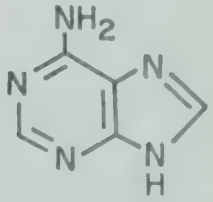
Williams, R. J., and Kirby, H., "Paper chromatography using capillary ascent," *Scie* 107, 481 (1948).

Date of issue: June 1960

Part of NAS-NRC Publication 719

Adenine [6-Aminopurine]

Formula: $C_5H_5N_5$
Molecular Wt.: 135.1



Usually available as free base and as salts (or hydrates of either).

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 13.1×10^3, at 262 mμ, 0.1 *N* HCl	
Ratio A 250/260 = 0.76 ± 0.02, 0.1 *N* HCl	± 2%
A 280/260 = 0.38 ± 0.02	
A 290/260 = 0.03 ± 0.01	

Literature References: Loring, H. S., Fairley, J. L., Bortner, H. W., and Seagran, H. L., *J. Biol. Chem.*, **197**, 809 (1952); E. Chargaff and J. N. Davidson (Editors), *Nucleic acids: chemistry and biology, Vol. I*, Academic Press, Inc., New York, 1955, pp. 200, 498.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **guanine** and **hypoxanthine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Source; knowledge of 6-methylaminopurine content.

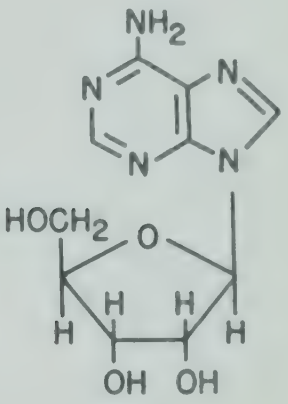
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Adenosine [9-β-D-Ribofuranosyladenine]

Formula: $C_{10}H_{13}O_4N_5$
Molecular Wt.: 267.2



Usually available as free nucleoside or its hydrates.

Spectral Reference Values (Tentative)

		Experimental variation*
a_M (Molar Absorbancy):	15.4×10^3, at 259 mμ, at pH 7.0 (M/15 phosphate buffer)	± 2%
Ratio A 250/260 =	0.78 ± 0.02, at pH 7.0	
A 280/260 =	0.15 ± 0.01	
A 290/260	<0.01	

Literature Reference: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **guanosine, adenine,** and **inosine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Source; rotation.

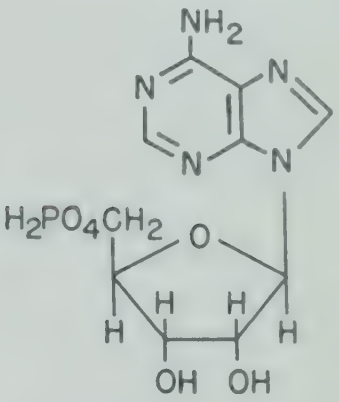
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Adenosine 5′-Phosphoric Acid (AMP)

Formula: $C_{10}H_{14}O_7N_5P$
Molecular Wt.: 347.2



Usually available as free acid and as salts (or hydrates of either).

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 15.4×10^3, at 259 mμ, at pH 7.0 (M/15 phosphate buffer)	± 2%
Ratio A 250/260 = 0.80 ± 0.02, at pH 7.0	
A 280/260 = 0.15 ± 0.01	
A 290/260 <0.02	

Literature Reference: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determinations of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **inosinic acid** and **adenosine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Source; inorganic ion content; knowledge of content of 2′- and 3′-phosphates.

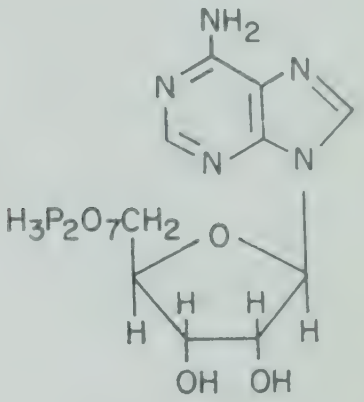
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Adenosine 5′-Pyrophosphoric Acid (ADP)

Formula: $C_{10}H_{15}O_{10}N_5P_2$
Molecular Wt.: 427.2



Usually available as hydrated salts.

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 15.4×10^3, at 259 mμ, at pH 7.0 (M/15 phosphate buffer) — ± 2%

Ratio A 250/260 = 0.78 ± 0.02, at pH 7.0
A 280/260 = 0.16 ± 0.01
A 290/260 <0.03

Literature Reference: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **ATP, AMP,** and other **nucleotides.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Pyrophosphates; orthophosphates; source; N, P analyses.

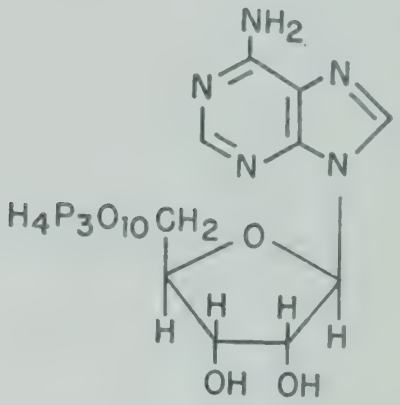
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Less than 5°; dry.

Date of issue: June 1960

Adenosine 5′-Triphosphoric Acid (ATP)

Formula: $C_{10}H_{16}O_{13}N_5P_3$
Molecular Wt.: 507.2



Usually available as hydrated salts.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 15.4×10^3, at 259 mμ, at pH 7.0 (M/15 phosphate buffer)	± 2%
Ratio A 250/260 = 0.78 ± 0.02, at pH 7.0	
A 280/260 = 0.15 ± 0.01	
A 290/260 <0.02	

Literature Reference: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **ADP, AMP,** and other **nucleotides.**

Additional Information Desirable:

Pyrophosphates; orthophosphates; source; N, P analyses.

Other pertinent information to be furnished at the discretion of the supplier.

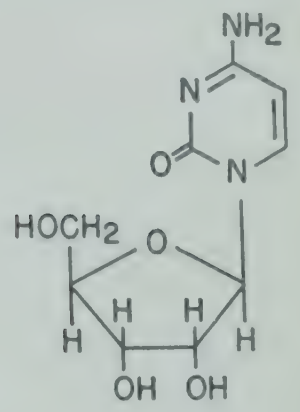
Supplementary Remark: Phosphate transferable to hexose by hexokinase would be desirable.

Storage: Less than 5°; dry.

Date of issue: June 1960

Cytidine [1-β-D-Ribofuranosylcytosine]

Formula: $C_9H_{13}O_5N_2$
Molecular Wt.: 243.2



Usually available as free nucleoside or its sulfate.

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 13.0×10^3, at 280 mμ, 0.1 *N* HCl — ± 2%

Ratio A 250/260 = 0.45 ± 0.02, 0.1 *N* HCl

A 280/260 = 2.10 ± 0.05

A 290/260 = 1.60 ± 0.04

Literature References: Loring, H. S., Fairley, J. L., Bortner, H. W., and Seagran, H. L., *J. Biol. Chem.*, **197**, 809 (1952); Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956); Fox, J. J., Yung, N., Wempen, I., and Doerr, I. L., *J. Am. Chem. Soc.*, **79**, 5060 (1957).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **uridine** and **cytosine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Source; rotation.

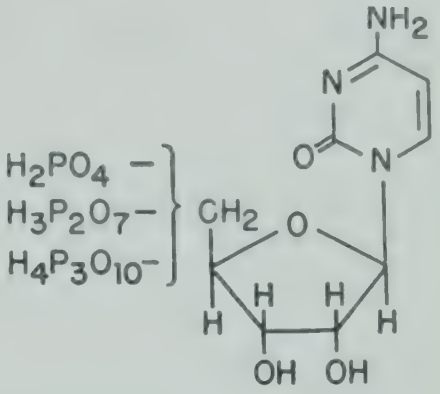
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Cytidine 5′-Phosphoric Acid (CMP)
Cytidine 5′-Pyrophosphoric Acid (CDP)
Cytidine 5′-Triphosphoric Acid (CTP)

Formulas:	Molecular Wts.:
CMP, $C_9H_{14}O_8N_3P$	323.2
CDP, $C_9H_{15}O_{11}N_3P_2$	403.2
CTP, $C_9H_{16}O_{14}N_3P_3$	483.2



Usually available as hydrated salts.

Spectral Reference Values (Tentative)

Experimental variation* ± 4%

a_M (Molar Absorbancy): 13.0×10^3, at 280 mμ, at pH 2

Ratio A 250/260 = 0.45 ± 0.04, at pH 2

A 280/260 = 2.10 ± 0.07

A 290/260 = 1.60 ± 0.05

Literature References: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956); Volkin, E., and Cohn, W. E., in D. Glick (Editor), *Methods of biochemical analysis, Vol. 1*, Interscience Publ., New York, 1954, p. 304.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement. Note: These values are an average of those reported for the individual compounds.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities, which are, for CMP: **UMP, AMP,** and **cytidine;** for CDP: **CMP, CTP,** and **UDP;** for CTP: **CDP, CMP,** and **UTP.**

Techniques and results are to be presented by the supplier.

Additional Information Desirable:

Orthophosphate; source; N, P analyses.

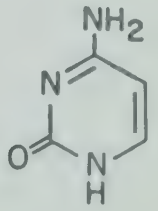
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Less than 5°; dry.

Date of issue: June 1960

Cytosine [4-Amino-2(1-H)-pyrimidinone]

Formula: $C_4H_5ON_3$
Molecular Wt.: 111.1



Usually available as free base.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 10.4×10^3, at 275 mμ, 0.1 *N* HCl	± 2%
Ratio A 250/260 = 0.48 ± 0.02, 0.1 *N* HCl	
A 280/260 = 1.50 ± 0.03	
A 290/260 = 0.77 ± 0.02	

Literature References: Hitchings, G. H., Elion, G. B., Falco, E. A., and Russell, P. B., *J. Biol. Chem.*, **177**, 357 (1949); Shugar, D., and Fox, J. J., *Biochim. et Biophys. Acta*, **9**, 199 (1952).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the most probable impurity: **uracil.**
Technique and results are to be presented by the supplier.

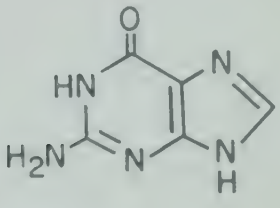
Additional Information Desirable:
Source.
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Guanine [2-Aminohypoxanthine]

Formula: $C_5H_5ON_5$
Molecular Wt.: 151.1



Usually available as free base and as salts (or hydrates of either).

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 11.4×10^3, at 248 mμ, 0.1 *N* HCl — ± 2%

Ratio A 250/260 = 1.37 ± 0.03, 0.1 *N* HCl

A 280/260 = 0.84 ± 0.02

A 290/260 = 0.49 ± 0.02

Literature Reference: Beaven, G. H., Holiday, E. R., and Johnson, E. A., in E. Chargaff and J. N. Davidson (Editors), *The nucleic acids; chemistry and biology, Vol. 1*, Academic Press, Inc., New York, 1955, p. 502.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **xanthine** and **adenine.** Guanine should be dissolved in 0.1 *N* HCl for application to paper.

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Source.

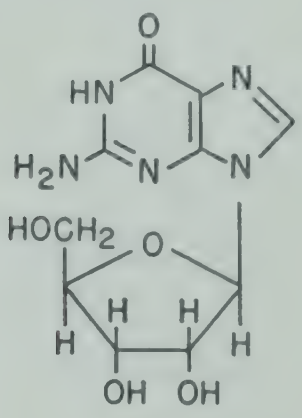
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Guanosine [9-β-D-Ribofuranosylguanine]

Formula: $C_{10}H_{13}O_5N_5$
Molecular Wt.: 283.2



Usually available as free nucleoside.

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 13.6×10^3, at 252.5 mμ, at pH 7.0 — ± 2%

Ratio A 250/260 = 1.15 ± 0.03, at pH 7.0

A 280/260 = 0.67 ± 0.02

A 290/260 = 0.27 ± 0.02

Literature Reference: Beaven, G. H., Holiday, E. R., and Johnson, E. A., in E. Chargaff and J. N. Davidson (Editors), *The nucleic acids: chemistry and biology, Vol. 1*, Academic Press, Inc., New York, 1955, p. 508.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **adenosine** and **guanine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Rotation.

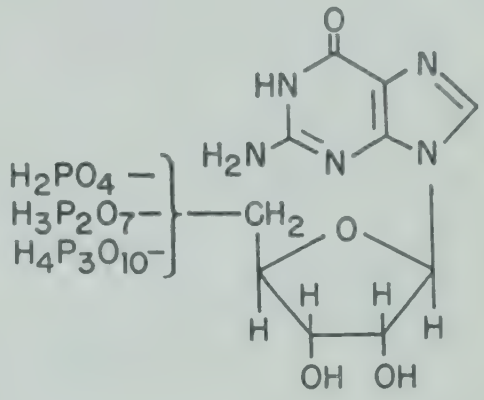
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Guanosine 5′-Phosphoric Acid (GMP)
Guanosine 5′-Pyrophosphoric Acid (GDP)
Guanosine 5′-Triphosphoric Acid (GTP)

Formulas:	Molecular Wts.:
GMP, $C_{10}H_{14}O_8N_5P$	363.2
GDP, $C_{10}H_{15}O_{11}N_5P_2$	443.2
GTP, $C_{10}H_{16}O_{14}N_5P_3$	523.2



Usually available as hydrated salts.

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 13.7×10^3, at 252 mμ, at pH 7 — ± 4%

Ratio A 250/260 = 1.16 ± 0.03, at pH 7

A 280/260 = 0.66 ± 0.02

A 290/260 = 0.28 ± 0.01

Literature References: Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956); Volkin, E., and Cohn, W. E., in D. Glick (Editor), *Methods of biochemical analysis, Vol. 1*, Interscience Publishers, New York, 1954, p. 304.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement. Note: These values are an average of those reported for the individual compounds.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities, which are, for GMP: **AMP, guanosine,** and **xanthylic acid;** for GDP: **GMP** and **GTP;** for GTP: **GMP** and **GDP.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

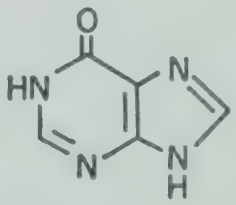
Orthophosphate; source; N, P analyses.

Storage: Less than 5°; dry.

Date of issue: June 1960

Hypoxanthine [6(1-H)-Purinone]

Formula: $C_5H_4ON_4$
Molecular Wt.: 136.1



Usually available as anhydrous compound.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 10.6×10^3, at 250 mμ, at pH 7.0	± 2%
Ratio A 250/260 = 1.32 ± 0.02, at pH 7.0	
A 280/260 = 0.09 ± 0.01	

Literature Reference: Beaven, G. H., Holiday, E. R., and Johnson, E. A., in E. Chargaff and J. N. Davidson (Editors), *The nucleic acids: chemistry and biology, Vol. 1*, Academic Press, Inc., New York, 1955, p. 499.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the most probable impurity: **adenine** (approximate per cent to be specified).
Technique and results are to be presented by the supplier.

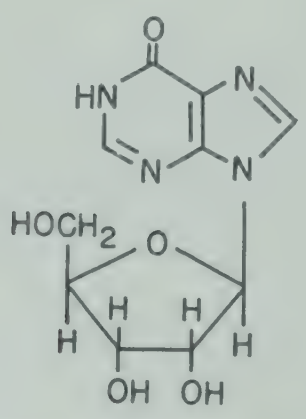
Additional Information Desirable:
If synthetic, analysis for absence of sulfur.
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Inosine [9-β-D-Ribofuranosylhypoxanthine]

Formula: $C_{10}H_{12}O_5N_4$
Molecular Wt.: 268.2



Usually available as anhydrous nucleoside or its hydrates.

Spectral Reference Values (Tentative) — Experimental variation*

a_M (Molar Absorbancy): 12.2×10^3, at 248.5 mμ, at pH 6.0 (M/15 phosphate buffer) ± 2%

Ratio A 250/260 = 1.73 ± 0.04, at pH 6.0
A 280/260 = 0.24 ± 0.02
A 290/260 = 0.02 ± 0.01

Literature Reference: Beaven, G. H., Holiday, E. R., and Johnson, E. A., in E. Chargaff and J. N. Davidson (Editors), *The nucleic acids: chemistry and biology, Vol. 1*, Academic Press, Inc., New York, 1955, p. 510.

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **hypoxanthine** and **adenosine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Rotation.

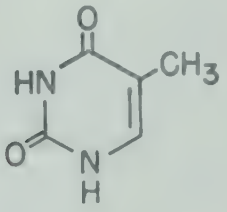
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Thymine [5-Methyluracil]

Formula: $C_5H_6O_2N_2$
Molecular Wt.: 126.1



Usually available as anhydrous compound.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 7.89×10^3, at 264.5 mμ, at pH 7.0	
Ratio A 250/260 = 0.67 ± 0.02, at pH 7.0	± 2%
A 280/260 = 0.53 ± 0.01	
A 290/260 = 0.09 ± 0.02	

Literature Reference: Shugar, D., and Fox, J. J., *Biochim. et Biophys. Acta*, **9**, 199 (1952).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities.
Technique and results are to be presented by the supplier.

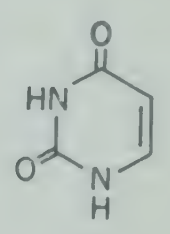
Additional Information Desirable:
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Uracil [2,4-(1H, 3H)-Pyrimidinedione]

Formula: $C_4H_4O_2N_2$
Molecular Wt.: 112.1



Usually available as anhydrous compound.

Spectral Reference Values (Tentative)

Experimental variation* ± 2%

a_M (Molar Absorbancy): 8.2×10^3, at 260 mμ, at pH 7.0 (M/15 phosphate buffer)

Ratio A 250/260 = 0.83 ± 0.02, at pH 7.0

A 280/260 = 0.20 ± 0.02

A 290/260 <0.02

Literature Reference: SHUGAR, D., AND FOX, J. J., *Biochim. et Biophys. Acta*, 9, 199 (1952).

(Values published at pH 7.2 in 0.015 M phosphate.)

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of impurities.

Technique and results are to be presented by the supplier.

Additional Information Desirable:

Test for absence of sulfur.

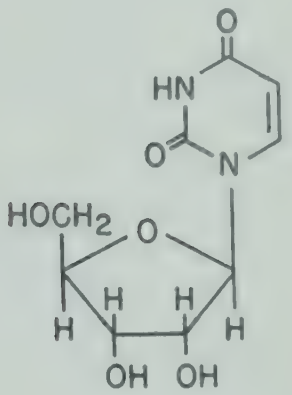
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: Juue 1960

Uridine [1-β-D-Ribofuranosyluracil]

Formula: $C_9H_{12}O_6N_2$
Molecular Wt.: 244.2



Usually available as anhydrous nucleoside.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 10.1×10^3, at 262 mμ, at pH 7.0	± 2%
Ratio A 250/260 = 0.74 ± 0.02, at pH 7.0	
A 280/260 = 0.36 ± 0.02	
A 290/260 <0.04	

Literature Reference: Fox, J. J., and Shugar, D., *Biochim. et Biophys. Acta*, 9, 369 (1952).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurity: **cytidine.** Technique and results are to be presented by the supplier.

Additional Information Desirable:

Rotation.

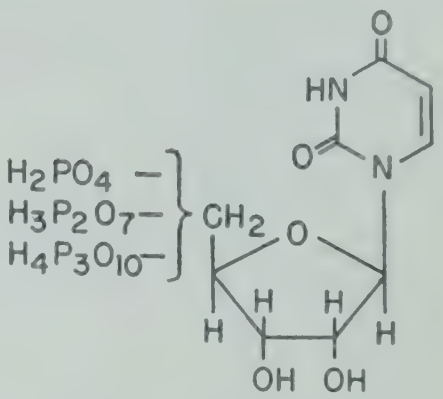
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Room temperature.

Date of issue: June 1960

Uridine 5′-Phosphoric Acid (UMP)
Uridine 5′-Pyrophosphoric Acid (UDP)
Uridine 5′-Triphosphoric Acid (UTP)

Formulas:	Molecular Wts.:
UMP, $C_9H_{13}O_9N_2P$	324.2
UDP, $C_9H_{14}O_{12}N_2P_2$	404.2
UTP, $C_9H_{15}O_{15}N_2P_3$	484.2



Usually available as hydrated salts.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 10.0×10^3, at 262 mμ, at pH 7.0	± 4%
Ratio A 250/260 = 0.74 ± 0.03, at pH 7.0	
A 280/260 = 0.38 ± 0.02	
A 290/260 <0.04	

Literature References: Ploeser, J. M., and Loring, H. S., *J. Biol. Chem.*, **178**, 431 (1949); Bock, R. M., Ling, N.-S., Morell, S. A., and Lipton, S. H., *Arch. Biochem. Biophys.*, **62**, 253 (1956).

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement. Note: These values are an average of those reported for the individual compounds.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities, which are, for UMP: **AMP** and **uridine**; for UDP: **UMP** and **UTP**; for UTP: **UMP** and **UDP**.

Additional Information Desirable:

Orthophosphate; source; N, P analyses.

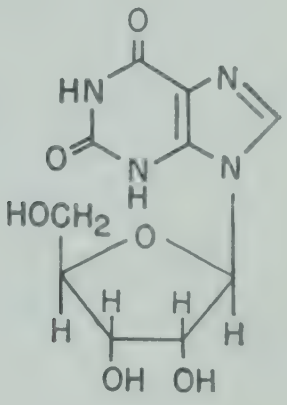
Other pertinent information to be furnished at the discretion of the supplier.

Storage: Less than 5°; dry.

Date of issue: June 1960

Xanthosine [9-β-D-Ribofuranosyl Xanthine]

Formula: $C_{10}H_{12}O_6N_4$
Molecular Wt.: 284.2



Usually available as dihydrate.

Spectral Reference Values (Tentative)

	Experimental variation*
a_M (Molar Absorbancy): 11.4×10^3, at 248.5 mμ, at pH 8.0	
Ratio A 250/260 = 1.31 ± 0.03, at pH 8.0	± 3%
A 280/260 = 1.13 ± 0.02	
A 290/260 = 0.61 ± 0.02	

Literature References: Unpublished data, obtained by members of the NAS-NRC subcommittee; BEAVEN, G. H., HOLIDAY, E. R., AND JOHNSON, E. A., in E. CHARGAFF AND J. N. DAVIDSON (Editors), *The nucleic acids: chemistry and biology, Vol. I*, Academic Press, Inc., New York, 1955, p. 508. (Beaven *et al.* report a_M values that appear to have been determined on a dihydrate.)

Spectral reference values have been selected from the literature and are tentatively considered to be the best presently available. The Committee on Biological Chemistry invites suggestions for revisions, particularly with reference to precise determination of a_M values.

* Estimated precision of a single measurement.

Homogeneity: Paper chromatographic evidence for purity should be presented from a minimum of two solvent systems that will permit the detection of small amounts of the more probable impurities: **xanthine, guanosine,** and **guanine.**

Technique and results are to be presented by the supplier.

Additional Information Desirable:

State of hydration; N analysis.

Other pertinent information to be furnished at the discretion of the supplier.

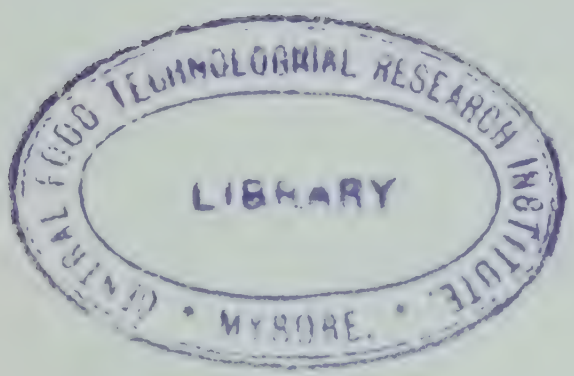
Storage: Room temperature.

Date of issue: June 1960

INDEX OF SUBJECT COMPOUNDS

This Index covers only compounds which are the subjects of *Specifications and Criteria* sheets. Other items (e.g., impurities, assay methods, etc.) are not indexed.

Numerals (1, 2, 3 . . . etc.), α, β, D, L, and comparable prefixes are ignored in alphabetizing, in the general manner of *Chemical Abstracts*. This Index lists all names and synonyms of subject compounds as given on their respective sheets. In addition, there are "inverted" entries whereby a stem or key name is brought forward to begin the listing, e.g., the subject compound of sheet C-7 is indexed both as 2-Deoxy-D-ribose (under D) and as D-Ribose, 2-deoxy- (under R).



Date of issue: June 1960

APPENDIX

Supplements, Orders, and Communications

This first edition of *Specifications and Criteria for Biochemical Compounds*, covering 113 biochemicals, represents a continuing project. Data for other compounds in the initial five categories and for compounds in additional categories are being compiled and will appear in Supplements, probably issued annually.

Supplement sheets for new compounds will continue the present code numbering; for example, the Carbohydrate section of the first Supplement will begin with C-34. Supplement sheets for each category of biochemicals may easily be integrated with existing pages, keeping old and new code numbers in sequence.

As better techniques are developed and better data obtained, revisions of existing compound sheets will be included in the Supplements. Revised sheets will retain their original headings and basic code numbers but will have a new "Date of issue," which will be an important part of the sheet's identification. Users are urged to discard replaced sheets or to mark them clearly as "Superseded."

A new and cumulative Table of Contents and a new and cumulative Index of Subject Compounds will be supplied with each major Supplement.

Price of the initial edition does not include a 3-ring binder nor subscription to Supplements. Purchasers will be notified when the first Supplement becomes available.

Additional copies of the first edition may be obtained by sending order and payment to:

Printing and Publishing Office
National Academy of Sciences—
National Research Council
2101 Constitution Ave., N.W.
Washington 25, D. C.

For payment with order, price is $1 to any destination in the United States and $1.50 for shipment to foreign countries. If a bill must be rendered, prices are $1.50 in the U. S. and $2 abroad. Payment may be by cash, check, money order, draft, or UNESCO coupons. Checks and money orders should be made payable to: "National Academy of Sciences."

It is stressed again that this publication represents "work in progress." Corrections, criticisms, and suggestions regarding both content and arrangement are requested and will be given earnest attention. Such communications should be sent to:

Committee on Biological Chemistry
Division of Chemistry and Chemical Technology
National Academy of Sciences—
National Research Council
2101 Constitution Ave., N.W.
Washington 25, D. C.

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